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EFFECT OF HERBIVORE INSECTS ON GROWTH AND REPRODUCTION OF *Cirsium arvense* IN ITS NATIVE AND INVASIVE RANGE

(Ph.D. thesis)

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I declare that this thesis has not been submitted for the purpose of obtaining the same or any other academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and manuscripts. All literature sources I used when writing this thesis have been properly cited.

In Prague, 2011

Inés Abela Hofbauerová

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SUMMARY

The presented Ph.D. thesis contains an introduction and four chapters concerning herbivory of *Cirsium arvense* in its native and invasive range. The chapters include two peer-reviewed papers and two manuscripts prepared for submission. The first chapter focuses on the effect of insect herbivory on plant growth and reproduction in field conditions. *C. arvense* in four populations in the Czech Republic were measured in 2005 and 2006 (chapter 1). Observational studies in *C. arvense* were done in the Czech Republic (Europe) and Nebraska (USA), native and invasive ranges, in 2006 and 2007. I tested whether plants suffer less damage than plants from populations in the native range, as the enemy release hypothesis (Keane, Crawley 2002) states (chapter 2). Afterwards I tested for direct and indirect effects of non-overlapping herbivore insects on plant growth and reproduction (chapter 3) and compared this effect between plants from the native (Spain and the Czech Republic, Europe) and invasive range (Nebraska and Illinois, North America). In a common garden experiment herbivore insects were added alone and in combinations to *C. arvense* which were planted in the Czech Republic in March 2008 and grew from seeds for two growing seasons (chapter 3). One underground insect (*Cleonis pigra*) and 3 aboveground insect species were used (*Cassida rubiginosa*, *Rhinocyllus conicus*, *Urophora cardui*). The last chapter is focused on *C. arvense* growth from the native and invasive range in experimental conditions, the evolution of increased competitive ability (EICA) hypothesis was tested.

The first chapter demonstrates that *C. arvense* experiences high levels of herbivory, with stem damage, flower herbivory and folivory having the strongest effects on plant performance. The evidence presented in the second paper confirms that in its native range, *C. arvense* experiences more plant damage and grows less than in the invasive range. Results from the third chapter show plants with herbivore addition grew less than plants without herbivores. The effect of combined insect was bigger than the single additions, suggesting a combination of more insects would be a better solution for biological control of *C. arvense*. From the results of the experimental study presented in the fourth chapter we sum up that plants from the invasive range grew more than the ones from the native range.

SOUHRN

Vliv Herbivorního hmyzu na růst a reprodukci Cirsium arvense v domácím a invazním areálu

Předkládaná disertační práce se skládá z obecného úvodu a čtyř kapitol, které spojuje téma vlivu herbivorního hmyzu na *Cirsium arvense* v domácím a invazním areálu. Kapitoly se skládají ze dvou publikací a dvou rukopisů připravených pro tisk. První kapitola se zaměřuje na vliv herbivorie hmyzu na růst a reprodukci rostlin v terenních podmínkách. Ve čtyřech populacích v České republice byl měřen růst a míra poškození *C. arvense* v letech 2005 a 2006 (1.kapitola). Studie *C. arvense* byly provedeny v České republice (Evropa) a Nebrasce (USA), v domácím a invazním areálu rostliny, v letech 2006 a 2007, čim jsem testovala, zda rostliny v invazním areálu rostou více a jsou poškozeny méně než rostliny v populacích v areálu domácím, jak prohlašuje enemy release hypothesis states (Keane, Crawley 2002) (2. kapitola). Následně jsem experimentálně testovala přímé a nepřímé vlivy nepřekrývajících se herbivorních druhů hmyzu na růst a reprodukci rostlin (3. kapitola) - porovnala jsem tento vliv mezi rostlinami v domácím (Španělsko a Česká republika, Evropa) a invazním areálu (Nebraska a Illinois, Severní Amerika). V zahradním experimentu v letech 2008-2009 byli přidáni zástupci herbivorního hmyzu individuálně a v kombinaci s rostlinami *C. arvense*, které byly vysázeny ze semen v České republice v březnu 2008. Byl použit jeden druh podzemního hmyzu (*Cleonis pigra*) a 3 druhy nadzemního hmyzu (*Cassida rubiginosa*, *Rhinocyllus conicus* a *Urophora cardui*). Poslední, čtvrtá kapitola je zaměřena na růst a reprodukci *C. arvense* v domácím a invazním areálu v experimentálních podmínkách, byla testovata hypotéza EICA (evolution of increased competitive ability).

Výsledky první kapitoly ukazují, že *C. arvense* má vysokou úroveň herbivorie, a poškození stonků, herbivorie úboru a folivorie mají nejsilnější vliv na růst rostliny. Důkazy předložené v druhé kapitole potvrzují, že v domácím areálu je druh *C. arvense* poškozen více a je menší, než v invazním areálu. Třetí kapitola ukazuje, že rostliny s přidaným herbivorním hmyzem rostly méně, než rostliny bez hmyzu. Pokud byli zástupci hmyzu přidáni do pokusu společně, měli na rostlinu větší vliv, než když byly jednotlivé druhy přidávány zvlášť. Tím poukazujeme na to, že kombinace více druhů herbivorního hmyzu, by byla pro kontrolu *C.arvense* lepší. Z výsledků experimentální studie prezentované ve čtvrté kapitole shrnujeme, že rostliny z invazního areálu rostly více, než rostliny z domácího areálu.

GENERAL INTRODUCTION

Understanding the impact of natural enemies on plant performance is one of the key issues when attempting to limit spread of invasive species. The aim of this Ph.D. thesis is to compare *Cirsium arvense* insect damage in its native and invasive range to uncover the effect of individual or combined insects on this weed. This work remains somewhere between insect-plant interactions, weed science, invasion biology and biological control.

Insect - plant interactions

More than 400 thousand phytophagous insect species live on 300 thousand vascular plant species (Schoonhoven et al. 2005). Polyphagous insects (generalist) feed on a wide range of plants and oligophagous or monophagous (specialists) feed on plants from the same family. Different groups of insects feed on different plant parts and are thus referred to as folivores, leaf-miners, gall-formers, stem-borers, root herbivores, flower-seed herbivores and fructivors.

Knowledge of insect-plant interactions goes far beyond the scientific interest because insect herbivory is an everlasting problem in agricultural production (Schoonhoven at al. 1991). The natural enemies of plants are also used as biocontrol agents to control spread of invasive species. Proper implementation of biological control, however, requires understanding of the effect of the natural enemies on plant performance as well as evaluation of the dangers of the escape of these natural enemies on other species (Howarth 1991, Simberloff and Stiling, 1996, Pemberton 2000, Louda 2002, 2003, Van Driesche et al. 2010).

Plant invasions from Europe to North America

With the European colonizations of North America in the 17th century many plants were introduced to the new world (Elton 2000). Some plant species got naturalized, and well established in crop fields and natural systems from North America. As the tens rule describes approximately ten percent of *species* pass through each transition from being imported to becoming *casual* to becoming established, and finally becoming a *weed* (Williamson, Brown 1986). Nowadays there are around 1500 invasive plant species in North America (web 1) and biological invasions are in second place as threat to biodiversity after direct habitat transformation (Soulé 1990) directly affecting agriculture and native biodiversity.

Herbivore insects as biological control for invasive plants

Biological control has several advantages over other types of weed control, sometimes offering the only solution (in protected areas) against alien plant invasions (Schoonhoven et al. 2005) and can be used in natural ecosystems as in crop fields.

Biological control of weeds has been used since 1881 to control invasive species (Delfosse 2000), and it is commonly used in North America, Australia, South Africa, Canada and New Zealand (McFadyen 1998).

It has been successful in cases such as *Opuntia stricta* management in Australia (Dodd 1940), and it is continuing to be successful in many examples (Caltagirone, 1981, McFadyden 1998) but the failure cases (cases when the control agents did not control the invasive weed and moreover it got invasive by itself) tells us to be careful (Louda 2002). Nevertheless, from 1200 cases of biological control worldwide, around 200 have proved completely successful (Bellows 2001).

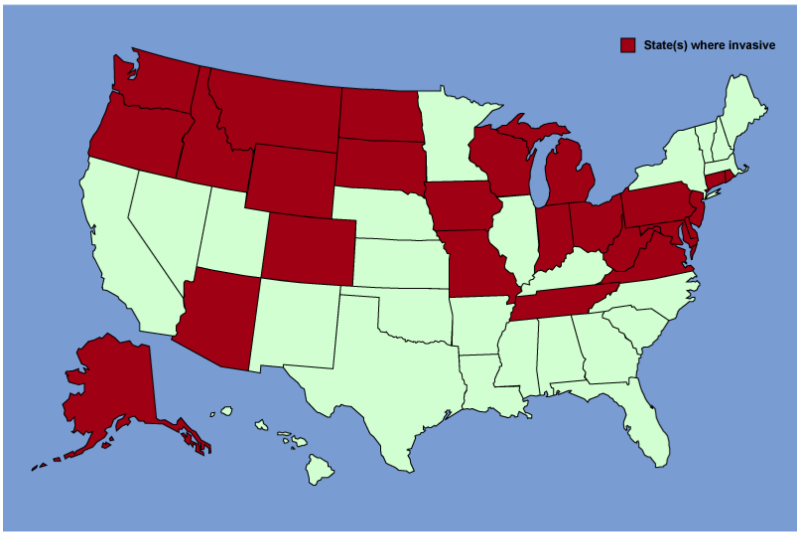
Herbivory common garden experiments vs. herbivory observational studies

Observational studies tell us the insect-plant natural composition in a certain population. It has the advantage of giving us the real interaction between plants and insects, but abiotic conditions are not very well controlled. These type of studies tell us whether plants in the invasive range escaped the insects (enemy release hypothesis), but do not tell us whether the difference in the herbivory damage is because the lack of the insects or because of the abiotic conditions. Because this type of studies are done in the field, only the effect of all insects toghether can be studied, not being able to separate the individual effect of each species.

On the other side, in common garden experiments, we can choose which abiotic conditions will the plant-insect system have. We can choose the type of substrate where the plants will grow (level of nutrients), the watering, we can control light by using various types of meshes on the plant, in greenhouse experiments the temperature can be adjusted. Nevertheless, the results of such experiments will be constrained by the conditions we choose. These experiments will test the EICA hypothesis, or how the plants perform without taking into account the abiotic conditions, and let us manipulate with various combinations of insect species.

Cirsium arvense as an invasive plant

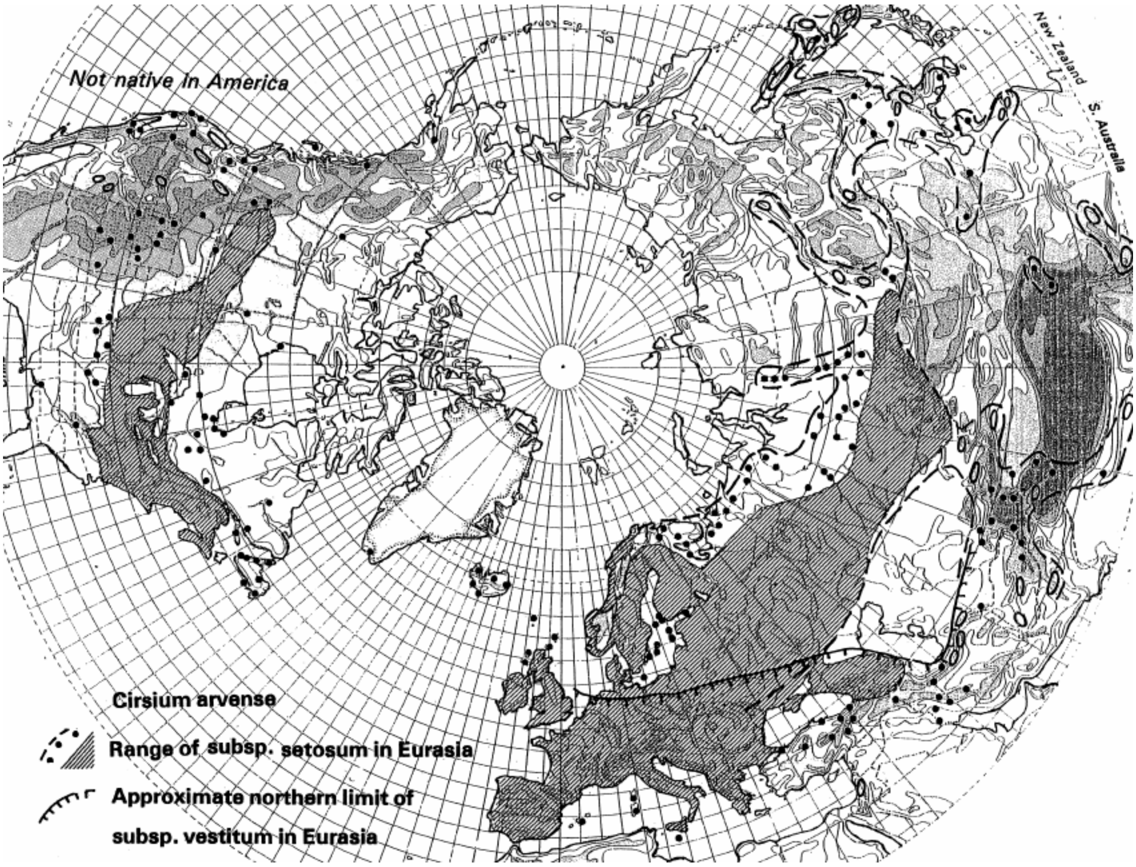
Cirsium arvense (L.) Scop (also *Serratula setosa* Willd., *Cirsium setosum* (Willd.) Bess., *C. setosum* (Willd.) Bieb., *Vreea setosa* (Willd.) Sojak) is the third most important weed in Europe (Schroeder *et al.* 1993), it is native to the South of Europe and is an archeophyte in many parts of Europe. It has however, been accidentally introduced to the new world around 1600 in North America and 130 years ago in New Zealand. It was believed to have arrived in North America via French settlements in the early 17th century (Dewey 1901) and the weed was recognized as a troublesome weed as early as 1795 when Vermont established legislation for its control (Moore 1975). Since then it got naturalized in many states of USA, Canada and New Zealand, becoming invasive in some, and being a focus for biological control in the last years.



States in USA where *Cirsium arvense* has an invasive status (web 1)

Use of Cirsium arvense as a tool for studying herbivory on invasive plants

Cirsium arvense hosts a wide range of herbivores including leaf-feeding, root crown and stem-boring, stem gall-forming and flower/seed feeding insects. One of the main insects causing leaf damage in *C. arvense* is *Cassida rubiginosa* (Mueller, 1776) (Coleoptera: Chrysomelidae). The main insect causing stem-boring is *Apion onopordi* (Kirby, W., 1808) (Coleoptera: Apionidae) (Friedli, Bacher 2001), *Urophora cardui* (Linnaeus, 1758) (Diptera: Tephritidae) causes stem galls, insects causing seed-head damage are *Larinus planus* (Fabricius, 1792) (Coleoptera: Curculionidae) and *Rhinocyllus conicus*. *Cleonis pigra* is a root-feeder. These species can be potentially used as a way of controlling their host plant, even though there is a need to study the plant-insect system for making sure we will not introduce one more invasive species when trying to control the plant invasive species.

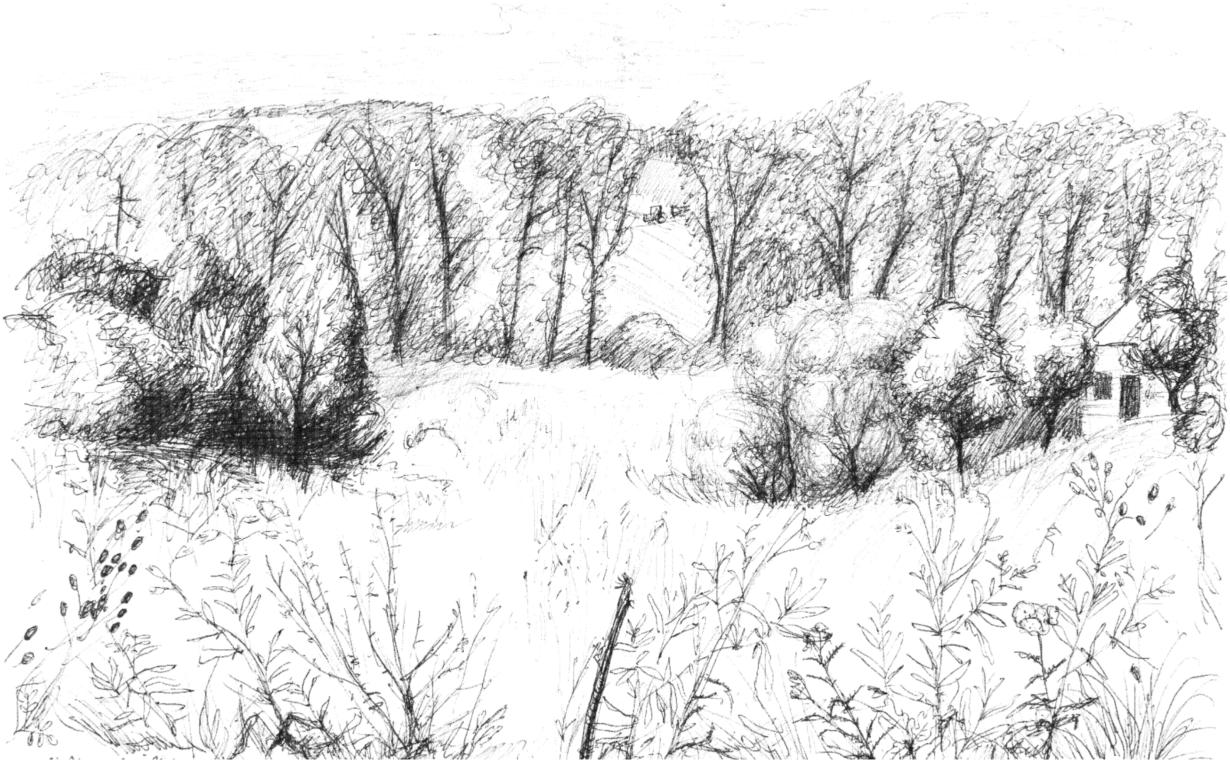


Distribution of *Cirsium arvense* in the northern hemisphere (Hulte and Fries, 1986). Reproduced with permission of Arne Anderberg.

Outline of the thesis

This thesis presents results of the research on effect of herbivore insects on *Cirsium arvense* in its native and invasive range. It consists of four chapters representing 4 independent manuscripts, from which two of them are already accepted for publication. The main goal of the thesis was to study an invasive plant in more detail, to uncover patterns of herbivore behavior on *C. arvense* (as one of many invasive plants in the new world) to be able to contribute with our new findings to improve the weed management in its invasive ranges in a more sustainable way.

Insect herbivory on plants may be approached by observational or experimental studies. The first gives us the option of choosing different habitats where a plant grows, so a wide range of abiotic conditions can be studied, but this can not be completely controlled. It tells us how much is a particular population or plant naturally damaged by a variety of herbivores. Observational studies had been addressed in the first chapter, in which *Cirsium arvense* natural occurring plant damage in its native range was studied. The results show folivory, external stem damage and leaf necrosis were most commonly found damages in *C. arvense* were and their occurrence varied between populations and years. Different plant damage types tended to be significantly associated with each other. It was therefore difficult to separate the effects of individual damage types and to study their interactions. *C. arvense* experiences high levels of herbivory, with stem damage, flower herbivory and folivory having the strongest effects on plant performance. Experimental studies need to confirm the results from chapter 1, which was performed in chapters 3 and 4.



The view from one Czech site when doing observational studies (Výžerky, drawing by Zita Červenková, 2006)

In the second chapter I compare natural occurring plant damages in plants in populations in the native and in the invasive range. I used data from Nebraska and the Czech Republic. The results show that plants in the invasive range possess less plant damage than in the native range and that *C. arvense* in the invasive range was higher than in the native range. Over all larger plants tend to be more damaged than smaller plants.

In the third chapter I compare *C. arvense* plant growth and insect damage from plants growing in the invasive and native range. I use representatives of 4 different insect guilds including flower head herbivores, folivores, gall-formers and root herbivores. The study showed that all the four tested insect species can reduce growth of *C. arvense* when added separately. Effect of the insects was significant only in some years and for some response variables. Surprisingly, the leaf feeding *C. rubiginosa* affected mainly flower head biomass and number of flower heads but had no effect on plant height and aboveground biomass. In contrast, the flower head feeding *R. conicus* had effect mainly on plant height and aboveground biomass. The gall forming *U. cardui* affected all the size measures. The root boring *C. pigra* significantly affected only plant height. All this indicates that the different insect species have a differential effect on various measures of plant performance. In addition, the different insects had effects in different years, in different substrates and for plants from different ranges.The effect of multiple insects on a single plant was generally stronger than expected from their simple addition. Then, a combination of multiple herbivores could be a better approach for controlling the plant than adding a single insect species as the insects seem to be largely complementary.

In the fourth chapter I move from studying *C. arvense* in native range natural populations to an experimental study with plants from the native and also from the invasive range. We do not focus on herbivory in this chapter, but compare plant growth from the native and invasive range in two types of substrate. The EICA hypothesis says plants in the invasive range grow more cause of being released from natural enemies (Blossey, Notzold 1995), and we test this hypothesis in the fourth chapter. The results confirm the EICA hypothesis, by finding plants from the invasive range grew more than plants from the native range. And we conclude that the differences in growth of plants between the native and the invasive range are genetically based.

Conclusion

The two main questions in my thesis proposal were i) which are the individual and combined effects of key insect herbivores to *C. arvense* plant and population growth in the native and invasive ranges and ii) how abiotic conditions as substrate nutrients can affect the interaction between the key insect herbivores in *C. arvense* in its native and invasive range.

I have answered to these main questions by a) performing plant damage and plant growth observational studies in both the native and invasive ranges (chapters 1 and 2) b) doing a factorial experiment, in which various types and combinations of insects herbivore that attack aboveground plant organs were added (chapter 3), c) performing a second experiment which approached the presence / absence of root herbivores alone and in combination with a set of aboveground insect herbivores(chapter 3), d) The third experiment compared the growth of plants from the native and invasive range grown from seeds under standard conditions(chapter 4). From observational and experimental studies we suggest growth and damage patterns can vary also depending on the level of nutrients in the substrate where plants grow, with the effect of insects being bigger in the poor substrate type, and also growing less in the poor substrate type.

I have found how certain insects affect *C. arvense* in both Europe and North America and in plants from those grown from both ranges in a controlled experiment in Europe. Because I found *C. arvense* experienced various plant damages and they tended to occur together in natural conditions in the native range (chapter 1), experimental studies were performed and by using insects alone and in combinations I wanted to answer whether the most effective herbivore hypothesis or the complementary hypothesis is the most suitable for choosing control agents for *C. arvense*. The results indicate that plants suffer more with combinations of insects more than individual addition (chapter 3) which support the complementary hypothesis, and suggest a combination of more than one insect could be used in biological control in *C. arvense*. We should be aware plant invasions happen in natural conditions and therefore study insect-plant and plant growth in the field. My finding suggest invasive plants have been released of insect herbivores in the invasive range (chapter 2), and maybe because for this reason *C. arvense* in the and from the invasive range grow better than from the native range (chapters 2 and 4).

I only studied one plant species, therefore the findings when testing the ERH, EICA, most effective herbivore and complementary hypothesis can be applied for *C. arvense*, but can not be applied for other plant species. Nevertheless, I suggest to weed science researchers to repeat the same way of approaching insect/s-invasive plant system I performed in my work so it becomes a rule for studies performed before biological control of weeds are carried out, therefore to help to avoid risks caused by non-target insect agents in the invasive range.

Even though this thesis quite clearly shows the impact that various insects have on *C. arvense*, we appeal that we do not directly recommend to use insect herbivores for the control of this weed and invasive plant, and that further studies are needed also in finding a combination of biological control and other sustainable agriculture measures.

References

Bellows T.S. (2001) Restoring population balance through natural enemy introductions. *Biological Control*, 21, 199-205.

Caltagirone L.E. (1981) Landmark examples in classical biological-control. *Annual Review of Entomology*, 26, 213-232.

Delfosse ES (2000) Forum—Biological Control: Important Tool for Managing Invasive Species*, Agricultural Research Magazine*, 48:3.

Dodd A. P. (1940) The biological campaign against prickly pear, Commonwealth Prickly Pear Board, Brisbane, Australia.

Friedli J. & Bacher S. (2001) Direct and indirect effects of a shoot-base boring weevil and plant competition on the performance of creeping thistle, *Cirsium arvense*. *Biological Control*, 22, 219-226.

Blossey, B. & Notzold, R., (1995) Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. *Journal of Ecology*, 83, 887-889.

Booth, B. D., S. D. Murphy & C. J. Swanton. (2003) Weed Ecology in Natural and Agricultural Systems. Cambridge, MA: CABI Publishing.

Biological Control of Invasive Plants in the United States
Eric M. Coombs, Janet K. Clark, Gary L. Piper, and Alfred F. Cofrancesco, Jr., Editors 2004. Corvallis, OR: Oregon State University Press.

Paul DeBach, David Rosen, Biological control by natural enemies

McFadyen R.E.C. (1998) Biological control of weeds. *Annual Review of Entomology*, 43, 369-393

Fagan W.F., Lewis M.A., Neubert M.G. & van den Driessche P. (2002) Invasion theory and biological control. *Ecology Letters, 5*, 148-157.

Howarth F.G. (1991) Environmental Impacts Of Classical Biological Control. *Annual Review of Entomology*, 36, 485-509.

Hultén, E. & Fries, M. 1986. Atlas of North European vascular plants: north of the Tropic of Cancer. - Koeltz Scientific Books, Königstein.

Louda S.M. & O'Brien C.W. (2002) Unexpected ecological effects of distributing the exotic weevil, Larinus planus (F.), for the biological control of Canada thistle. *Conservation Biology*, 16, 717-727.

Louda S.M., Pemberton R.W., Johnson M.T. & Follett P.A. (2003) Nontarget effects - The Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, 48, 365-396.

Pemberton R.W. (2000) Predictable risk to native plants in weed biological control. *Oecologia*, 125, 489-494.

Simberloff D. & Stiling P. (1996) Risks of species introduced for biological control. *Biological Conservation*, 78, 185-192.

L. M. Schoonhoven,J. J. A. van Loon & Marcel Dicke (2005) Insect-plant biology: Oxford University Press.

L. M. Schoonhoven,, Blaney WM. & Simmonds MS. J. (1991) European science foundation: Network on insect-plant interactions, *Chemoecology*, 2:1.

Soule M.E. (1990) The onslaught of alien species, and other challenges in the coming decades. *Conservation Biology*, 4, 233-239.

Elton C.S. (2000) The ecology of invasions by animals and plants, The University of Chicago Press.

Van Driesche R.G., Carruthers R.I., Center T., Hoddle M.S., Hough-Goldstein J., Morin L., Schroeder D., Muellerschaerer H. & Stinson C.S.A. (1993) A european weed survey in 10 major crop systems to identify targets for biological-control. *Weed Research*, 33, 449-458

Smith L., Wagner D.L., Blossey B., Brancatini V., Casagrande R., Causton C.E., Coetzee J.A., Cuda J., Ding J., Fowler S.V., Frank J.H., Fuester R., Goolsby J., Grodowitz M., Heard T.A., Hill M.P., Hoffmann J.H., Huber J., Julien M., Kairo M.T.K., Kenis M., Mason P., Medal J., Messing R., Miller R., Moore A., Neuenschwander P., Newman R., Norambuena H., Palmer W.A., Pemberton R., Panduro A.P., Pratt P.D., Rayamajhi M., Salom S., Sands D., Schooler S., Schwarzlander M., Sheppard A., Shaw R., Tipping P.W. & van Klinken R.D. (2010) Classical biological control for the protection of natural ecosystems. *Biological Control*, 54, S2-S33.

Hultén, E. & Fries, M. 1986. Atlas of North European vascular plants: north of the Tropic of Cancer. - Koeltz Scientific Books, Königstein.

Web 1: http://www.nps.gov/plants/alien/map/ciar1.htm

Williamson M.H. & Brown K.C. (1986) The analysis and modeling of british invasions. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, 314, 505-522.

CHAPTER 1

The effect of different natural enemies on the performance of *Cirsium arvense* in its native range

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The effect of different natural enemies on the performance of *Cirsium arvense* in its native range

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Summary

Understanding the effects of herbivores on weedy plant performance under different habitat conditions may provide useful information for limiting the spread of these plant species in their native and invaded ranges. A critical drawback to using herbivores to limit the spread of weedy plant species is the limited knowledge regarding the effects of different natural enemies on plant performance under natural conditions and in different habitat types. The aim of this study was to collect information on the degree and types of damage caused by natural enemies and their effects on the performance of the weed species *Cirsium arvense*, under different abiotic conditions in its native range in the Czech Republic. Damage induced by different natural enemies of *C. arvense* in four different populations in wet and dry sites was studied. The most common types of damage found in *C. arvense* were folivory (eaten foliage), external stem damage and leaf necrosis, and their occurrence varied strongly in space and time. Different plant damage types tended to be significantly associated with each other. It was therefore difficult to separate the effects of individual damage types and to study their interactions. Overall, the results indicate that in its native range, *C. arvense* experiences high levels of herbivory, with stem damage, flower herbivory and folivory having the strongest effects on plant performance. Experimental studies that would separate the effect of single herbivores are, however, needed to confirm this.

Keywords: creeping thistle, Canada thistle, herbivory, noxious species, plant damage, water, plant growth.

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Introduction

Understanding the effects of herbivores on weedy plant performance under different habitat conditions can provide useful information for limiting the spread of these plant species in their native and invasive ranges (e.g. Delfosse, 2000; Louda & O'Brien, 2002; Coombs *et al*., 2004). Interactions between plants and their insect herbivores have been the subject of many previous studies (e.g. Rhoades, 1985; Price, 1991; Münzbergová, 2006). Most of these studies, however, only examined a single insect species or a set of species causing the same type of damage (e.g. Hatcher *et al*., 1995; Bacher & Schwab, 2000; Friedli & Bacher, 2001). Only a few studies have investigated how damage from multiple insect species that typically occur in the field under natural conditions can affect plant growth (e.g. Hufbauer & Root, 2002).

When studying the effects of herbivores under natural conditions, it is also important to evaluate the effect of

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environmental conditions on this interaction. Two different hypotheses have been proposed to explain the effect of site conditions on plant responses to herbivores. The Vigour Hypothesis claims that plants from the most vigorous populations, the most vigorous plants within a population and/or the most vigorously growing parts within a plant, suffer higher herbivore damage (Price, 1991). This corresponds to the hypothesis of Cebrián and Duarte (1994), which asserts that herbivory pressure increases with increasing plant growth rate. The alternative Plant Stress Hypothesis predicts that more stressed plants will suffer greater damage from herbivores (White, 1974; Rhoades, 1985). These two hypotheses should not be considered mutually exclusive, and both mechanisms can apply in any single case (Price, 1991). Waring and Cobb (1992) suggested that observational studies usually support the Plant Stress Hypothesis, while experimental studies usually support the Vigour Hypothesis, but exceptions to this pattern exist (e.g. Cobb *et al.*, 1997).

In this study, we investigated *Cirsium arvense* (L.) Scop. (creeping thistle), which is one of the most problematic weeds in its invasive range in North America (Moore, 1975) and Australia (Rahman, 1982). This species is also an important weed in many parts of Europe, where it is considered to be native (Kasahara, 1982). *Cirsium arvense* has been the subject of many studies that have examined the diversity of phytophagous insects (e.g. Story *et al.*, 1985) and the effect of insects on its performance (e.g. Ang *et al.*, 1994; Friedli & Bacher, 2001) in its invasive range. In contrast to the large number of studies performed in the invasive range, comparatively few studies have examined the effect of insects on the species in its native range. Most of these studies are experimental (e.g. Bacher & Schwab, 2000; Friedli & Bacher, 2001; Skuhrovec *et al.*, 2008), and the field still lacks observational studies on the effect of insects on plant growth under natural conditions in different habitat types.

The specific aim of this study was to collect information on the degree and types of damage present on *C. arvense* under different abiotic conditions and in different years within its native range in the Czech Republic. We also assessed the effects of the different damage types on plant performance in the field within a single growing season. Specifically, we asked the following questions: (i) what types of damage from different natural enemies can be found on *C. arvense*? (ii) what types of damage tend to co-occur on a single plant? (iii) does the occurrence of different damage types caused by different natural enemies depend on abiotic conditions and plant size? (iv) what are the effects of different types of damage on plant growth? and (v) how do the effects vary between years and habitat types?

Materials and methods

Study system

The studied species was *Cirsium arvense*, a plant that is an herbaceous, perennial member of the Asteraceae family, with erect stems 0.5–1.0 m tall, prickly leaves and an extensive creeping rootstock. It is dioecious and reproduces by clonal growth. In Europe, it grows in cultivated ground, waste places, pastures and open woodlands (Tutin *et al.*, 1976). Despite its North American name, Canada thistle, it is native to Europe, parts of North Africa and Asia, including Afghanistan, Iran, Pakistan and China. This species was introduced to North America and Australia from Europe in the 1600s as a contaminant of grain seeds being transported by ships (Rahman, 1982).

Natural enemies of C. arvense

Eleven types of damage, caused by a wide range of natural enemies, were previously reported in *C. arvense*, including leaf cocoons, necrosis in leaves, folivory (foliage eaten), leaf mines, stem galls, other external stem damage, flowerhead necrosis, flowerhead herbivory, herbivory of the shoot top, as well as damage caused by rust fungus and spittle-bugs (Table 1).

Leaf damage can lead to leaf necrosis, causing greater leaf damage than that caused directly by the feeding insects. Cocoons on leaves are built in *C. arvense* by a wide range of spiders and lead to another type of leaf damage and the reduction of the photosynthetically active area of the leaf (I Abela Hofbauerová, pers. obs.). Similar to other leaf damage, presence of leaf mines can also later lead to leaf necrosis.

Study area

Two different localities in Central Bohemia, Czech Republic, Europe, were selected in spring 2005. Locality 1 is situated by the village of Kytín (460 m a.s.l., N 49° 51′ 06″, E 14° 12′ 37″). Locality 2 is situated by the village of Vyžerky (340 m a.s.l., N 49° 56′ 56″, E 14° 53′ 95″). At each locality, two sites were selected on a moisture gradient: one site was next to a stream and the other was approximately 300 m farther away in a dryer area. We refer to the two sites within a locality as the wet and dry sites respectively.

Experimental design

At each site, 300 plants were selected in spring 2005. Plants in each site were marked within 10 experimental plots delineated with four sticks surrounded by a string.

Table 1 Identity of insect herbivores likely to cause types of plant damage to *Cirsium arvense*. There are 38 listed phytophagous insects for *C. arvense* and 83 associated fungi in Europe [Fitter, Peat (1994) The Ecological Flora Database, *Journal of Ecology* **82**, 415–425, http://www.ecoflora.co.uk]

Stem damage	
Monophagous insects	
Hemiptera: Aphididae	*Uroleucon cirsii* (Linnaeus, 1758)
Diptera: Tephritidae	*Urophora cardui* (Linnaeus, 1758)
Lepidoptera: Tortricidae	*Aethes cnicana* (Westwood, 1854) *Epiblema scutulana* (Denis, Schiffermüller, 1775)
Oligophagous insects	
Hemiptera: Aphididae	*Aphis fabae* Scopoli 1763, *Brachycaudus cardui* (Linnaeus, 1758) *Dysaphis lappae* (Koch, 1854) *Uroleucon aeneus* (Hille Ris Lambers, 1939)
Hemiptera: Cercopoidea	Spittle-bugs
Coleoptera: Apionidae	*Ceratapion carduorum* (W. Kirby, 1808)
Coleoptera: Curculionidae	*Cleonus pigra* (Scopoli, 1763)
Diptera: Agromyzidae	*Melanagromyza aeneoventris* (Fallén, 1823)
Leaf damage	
Monophagous insects	
Hemiptera: Aphididae	*Uroleucon cirsii* (Linnaeus, 1758)
Lepidoptera: Crambidae	*Anania perlucidalis* (Hübner, 1809)
Oligophagous insects	
Hemiptera: Aphididae	*Capitophorus elaeagni* (Del Guercio, 1894) *Uroleucon aeneus* (Hille Ris Lambers, 1939)
Hemiptera: Cicadellidae	*Eupteryx notata* (Curtis, 1837)
Coleoptera: Chyrsomelidae	*Cassida rubiginosa* Müller, 1776
Coleoptera: Curculionidae	*Hadroplontus litura* (Fabricius, 1775)
Diptera: Agromyzidae	*Phytomyza autumnalis* Hering, 1957
Lepidoptera: Coleophoridae	*Coleophora paripennella* Zeller, 1838
Lepidoptera: Depressariidae	*Agonopterix arenella* (Denis, Schiffermüller, 1775)
Lepidoptera: Gelechiidae	*Scrobipalpa acuminatella* (Sircom, 1850)
Leaf mines	
Diptera: Agromyzidae	*Phytomyza autumnalis* Hering, 1957 (pers. obs.)
Seed-head damage	
Coleoptera: Curculionidae	*Larinus planus* (Fabricius, 1792) *Rhinocyllus conicus* (Frölich, 1792)
Rust	
Fungi: Pucciniales	*Puccinia punctiformis* [(F. Strauss) Röhl., 1813]

All of the plants in each plot were marked, and there were from 13 to 70 plants per plot. The plants were marked with a plastic, visible mark and one metal mark in the ground, both carrying an identical plant number (the latter could be used to identify the plant if the plastic mark were lost). The size of the plots varied between 1 and 2 m^2, depending on plant density. Plots within these sites were all situated within 100 m among patches of *C. arvense*. Originally, we wanted to study the same plants in both years. Because of extensive clonal growth, however, the plants appeared at different places in different years. Therefore, we had to move the labels in the second spring and could not trace the plants in the first year to the same plants in the second year. We were thus not able to tell whether the plants studied 1 year were the same clones as those observed the next year. In all of the analyses, we considered the plants from the different years to be independent of each other. In total, 10 plots × 2 sites (wet and dry) × 2 populations were measured, with 13–70 plants per plot and two seasons of measurements. In total, 3092 plants were studied over the 2 years.

Measurements of plant performance and plant damage

Each ramet was measured in early, mid and late season. The early season measurements were made from the end of April to May in 2005 and 2006. We measured traits describing plant performance: basal stem diameter (measured using a digital calliper), number of leaves and length of the longest leaf.

The mid-season measurements were made in July 2005 and 2006. During this period, we measured plant performance and plant damage caused by different natural enemies. The data on plant performance included the height of each ramet, the number of leaves and the length of the longest leaf in the vegetative ramets, the number of flowering branches per plant and number of flowerheads in every third flowering branch in the flowering ramets.

To quantify the damage, we recorded the presence of cocoons and spittle-bug spit on each plant, approximate % of plant folivory, approximate % of leaf mines, number of stem galls per plant (and their length and width) and flowerhead damage (estimated as the proportion of flowerheads in the inflorescence with external evidence of herbivory and necrosis). Separately, we recorded herbivory of the upper parts of the inflorescence, i.e. one or more terminal flowers, referred to as herbivory of the shoot top. We also recorded the approximate % of rust per plant, % stem damage and % necrosis in the leaves. Damage caused by spittle-bugs and rust (*Puccinia punctiformis*) can be very easily determined. All of the other damage types are described without specific reference to the insect causing the damage. The possible agents causing these different damage types will be discussed in the following sections.

The end season, measurements were made in September 2005 and 2006. At this time, we measured the basal stem diameter for the second time in the season. We calculated growth, determined as changes in basal stem diameter between spring and autumn.

Data analyses

Plant damage was quantified by estimating the proportion of the plant that had suffered damage. Originally, we assumed that we would find a gradient of plant damage, from plants with little damage to plants with severe damage. In our data set, however, plant damage showed a bimodal distribution, as the plants were generally not damaged at all or damaged. For the analyses, percentages were therefore converted to the presence/absence of plant damage.

To test whether the different plant damage types tend to be associated with each other within single plants, we performed a pairwise analysis using pivot tables and a chi-square test, combining data from both years together.

Factors determining the occurrence of individual plant damage caused by natural enemies were studied using logistic regression in S-PLUS (2000). Specifically, we tested the effect of year, number of leaves per plant in the spring (a measure of plant size), locality and site conditions (wet, dry) for the two seasons on the occurrence of individual natural enemies.

To study the effects of different natural enemies on plant growth, we first performed a logistic regression in which we used one type of damage, year, locality, abiotic conditions (dry/wet) and their interactions as independent variables. Then, we used stepwise linear regression with all plant damage types, year, locality, abiotic conditions (dry/wet) and their interactions as independent variables. Significant plant damage types were selected using both directional stepwise selections. We also wanted to elucidate the effect of interactions between single plant damage types. Occurrence of different damage types was, however, so strongly correlated that this test was not possible. Afterwards, we tested the effects of the number of different damage types per plant, year, locality, abiotic conditions (dry/wet) and their interactions as independent variables. The dependent variables in all cases were either basal stem diameter in the autumn or the number of flowerheads per plant. Basal stem diameter in the spring was used as a covariate in both cases to take into account plant size differences at the beginning of the field season. For these analyses, we used a generalised linear model with gamma distribution. Owing to overdispersion of the data, we used F test for the test statistics.

For all statistical tests, we considered each measured plant as an independent replicate. Strictly speaking, however, we had only two replicates (localities) for testing the effects of habitat conditions. This low number of replicates prevented us from identifying any patterns. Our two localities and 2 years therefore aided us by covering all possible spatial and temporal variations in the area and were not considered as tested units.

Results

Basal stem diameter, number of leaves per plant and number of flowerheads per plant were not significantly different between plants from the two habitat types or between years ($P > 0.1$ in all cases). This indicates that the possible differences in plant–herbivore interactions between habitat types and years are not because of differences in plant size.

Occurrence of damage

The three most common plant damage types in *C. arvense* were folivory (52% of plants), stem damage (36%) and leaf necrosis (33%) (Table 2). These were followed by mines, flowerhead herbivory, rust fungus, flowerhead necrosis and herbivory of the shoot top. Stem galls, cocoons on leaves and spittle-bug spit at the base of the stem were rare (Table 2). In pairwise analyses of co-occurrence, there were many significant correlations between different pairs of damages (Table 3).

Factors determining occurrence of individual damage types

Leaf necrosis observed in summer was more common in plants with fewer leaves in the spring (Fig. 1A). On the other hand, folivory, stem damage, herbivory of the shoot top and flowerhead herbivory observed in summer were more common in plants with more leaves in the spring (Table 4, Fig. 1B,C). The occurrence of other types of damage was independent of plant size in the spring (Table 4). Leaf necrosis, folivory and stem damage were more common in locality 1, and mines, galls and flowerhead herbivory were more common in locality 2 (Table 4).

Flowerhead herbivory was more common in wet sites. The occurrence of other damage types was independent of abiotic conditions at the site (Table 4). The occurrence of all damage types, except for rust, depended on the year. All of the damage types, except

Table 2 Percentage of plants with a given damage type in the first and second years, in both wet and dry sites at the two studied localities

Damage types	% damaged plants in 1st year				% damaged plants in 2nd year			
Abiotic conditions	Wet		Dry		Wet		Dry	
Locality	1	2	1	2	1	2	1	2
Folivory	6.4	83.8	66.2	20.9	83.1	34.5	40.9	81.0
Stem damage	0.0	63.6	41.9	16.4	61.9	3.4	30.8	69.3
Leaf necrosis	0.0	75.7	14.9	12.7	16.1	83.9	4.4	54.6
Mines	0.0	37.1	0.0	0.0	1.4	5.1	5.4	4.5
Flowerhead herbivory	0.0	26.8	29.7	1.5	0.8	23.2	6.3	2.5
Rust	0.0	12.5	0.0	0.4	23.7	26.2	12.6	13.5
Flowerhead necrosis	0.0	12.5	0.0	0.4	23.7	26.2	12.6	13.5
Herbivory of shoot top	0.7	14.0	13.5	4.5	0.0	1.5	1.4	1.5
Galls	0.0	1.5	1.4	1.5	6.8	14.3	1.3	6.1
Cocoons	0.0	2.9	2.7	1.1	12.7	11.9	2.5	5.5
Spittle-bugs	0.0	0.0	0.0	0.0	5.1	12.5	0.6	4.9

Table 3 Pairwise co-occurrence of the different damage types in marked plants of *Cirsium arvense* for the first and second seasons, analysed using the chi-square test

	Pairwise combination of plant damage types								
	Folivory	Mines	Rust	Stem damage	Leaf necrosis	Flowerhead herbivory	Herbivory of shoot top	Top necrosis	Cocoons
Folivory		+	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mines	+		+	+	+	n.s.	+	n.s.	+
Rust	n.s.	+		−	+	n.s.	+	+	+
Stem damage	n.s.	+	−		−	+	+	+	+
Leaf necrosis	n.s.	+	+	−		n.s.	+	n.s.	+
Flowerhead herbivory	n.s.	n.s.	n.s.	+	n.s.		+	+	+
Herbivory of shoot top	n.s.	+	+	+	+	+		+	+
Top necrosis	n.s.	n.s.	+	+	n.s.	+	+		+
Cocoons	n.s.	+	+	+	+	+	+	+	

The direction of the relationship is given for significant co-occurrences ($P \leq 0.05$). n.s. indicates non-significant relationships with $P > 0.05$. The number of studied plants (N) = 3092.

for leaf necrosis, galls and flowerhead herbivory, were more common in the second year (Tables 1 and 3).

Effect of natural enemies on growth of C. arvense

When analysing the effects of individual damage types on plant growth (measured as basal stem diameter in autumn after using the effect of plant size in spring as a covariate), leaf necrosis, folivory, stem damage, herbivory of the shoot top, rust and mines had significant negative effects on plant growth (in the column 'Single' in basal stem diameter, Table 5). There was also a significant interaction between year and the effect of damage for leaf necrosis, herbivory of the shoot top and mines (in the column 'Single' in basal stem diameter, Table 5). The interactions between the effects of damage and abiotic conditions were significant for leaf necrosis, folivory, stem damage, herbivory of the shoot top, rust and mines.

Growth of plants with stem damage (Fig. 2A), leaf necrosis, mines and rust (data not shown) was more notably suppressed in wet than in dry sites. In contrast, growth of plants with folivory (Fig. 2B) and damage of the shoot top (data not shown) was more suppressed in dry sites. When all damage types were included in the model, only stem damage, which reduced plant growth by 11.8%, measured by basal stem diameter, was significant (in the column 'Step' in basal stem diameter, Table 5).

When analysing the effects of individual damage types on the number of flowerheads in the summer, after removing the effect of plant size in the spring, leaf necrosis, folivory, stem damage, flowerhead herbivory, rust and mines had significant negative effects on plant growth (in the column 'Single' in No. of flowerheads, Table 5). Abiotic conditions and year were also significant. The number of flowerheads in plants with leaf necrosis, rust or mines was higher in the first year (in the column 'Single' in No. of flowerheads, Table 5, row damage × year). It was also higher in plants with folivory in dry sites (in the column 'Single' in No. of

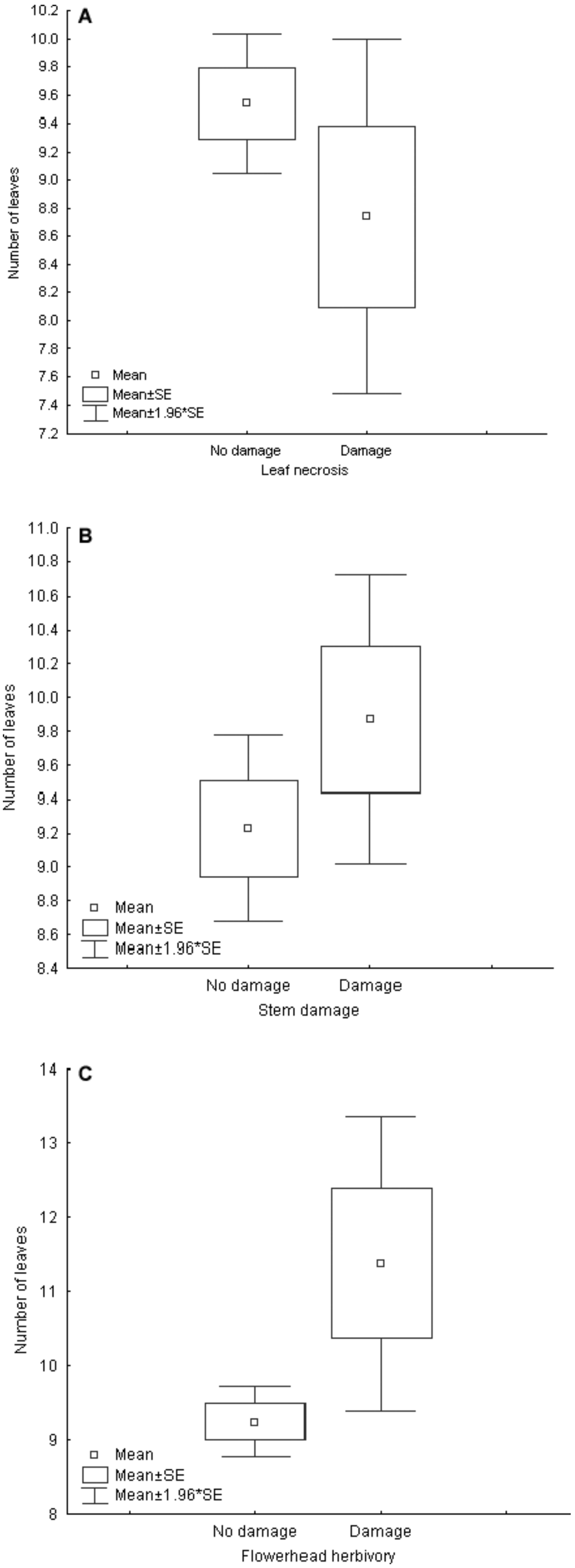

Fig. 1 Occurrence of (A) leaf necrosis, (B) stem damage and (C) flowerhead herbivory in the summer, relative to the number of leaves on the plant in the spring.

flowerheads, Table 5, row damage × abiotic conditions). When we included all damage types in the model, we found that folivory, stem damage and herbivory of the shoot top affected the number of flowerheads per plant in the summer (in the column 'Step' in No. of flowerheads, Table 5). The number of flowerheads was reduced 74.4% by folivory, 65.3% by stem damage and 81.1% by herbivory of the shoot top.

The number of damage events per plant did not have any significant effect on plant growth, as measured by changes in basal stem diameter ($P = 0.085$). There was, however, a significant interaction between the number of damage events and the year ($P < 0.001$), indicating that the negative effects of the number of plant damage events were stronger in the first than in the second year (Fig. 3A). The number of damage events per plant also did not have a significant effect on plant growth, measured as the number of flowerheads in the summer (after taking into account basal stem diameter in the spring, $P = 0.099$). There was, however, a significant interaction between the number of damage events and abiotic conditions ($P < 0.001$), indicating that the negative effects of the number of plant damage events were stronger in wet than in dry sites ($P = 0.036$, Fig. 3B).

Discussion

Different types of plant damage often co-occur within a single plant, making it quite difficult to separate the effects of individual types of damages and to study the interactions between them. Stem damage significantly affected plant growth, measured as the change in basal stem diameter. Stem damage, folivory and herbivory of the shoot top had significant negative effects on plant reproduction, measured as the number of flowerheads per plant. The damaged plants had 11.8% smaller basal stem diameter and up to 80% fewer flowerheads than the undamaged plants, suggesting that the presence of natural enemies can have strong long-term consequences on plant performance.

One of the most common types of damage observed in our study was stem damage. Frequent occurrence of stem damage was also observed in a study by Freese (1993), supporting the observation that insects that cause stem damage are common herbivores of *C. arvense*. In our studied sites, external stem damage was probably caused by gall-forming insects, such as *Urophora cardui*, because stem galls and external stem damage often co-occurred on single plants. Additional stem damage can be attributed to *Cleonus pigra* (Scopoli, 1763) (Coleoptera: Curculionidae), which commonly occurs at the studied localities. Another common damage type recorded in our study was folivory, probably caused by *Cassida rubiginosa*

Table 4 Factors determining the occurrence of individual types of plant damage caused by natural enemies and assessed using logistic regression

	Leaf necrosis	Folivory	Stem damage	Herbivory of shoot top	Rust	Mines	Cocoons	Galls	Flowerhead herbivory	Top necrosis
No. leaves	0.118 –	0.084 +	0.091 +	0.14 +	n.s.	n.s.	n.s.	n.s.	0.095 +	n.s.
Locality	0.153 +	0.031 +	0.05 +	0.010	n.s.	0.049 –	n.s.	0.023 –	0.068 –	n.s.
Abiotic cond.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.078 –	n.s.
Year	0.035 –	0.141 +	0.158 +	0.049 +	n.s.	0.159 +	0.078 +	0.052 –	0.078 –	0.187 +
No leaves × Locality	n.s.	n.s.	n.s.	0.084 –	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Abiotic cond. × No. leaves	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Abiotic cond. × Locality	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Abiotic cond. × Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Locality × Year	n.s.	n.s.	n.s.	n.s.	0.030	n.s.	n.s.	n.s.	0.008	n.s.
No. leaves × Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

The values are R^2 values for significant relationships ($P \le 0.05$). No. leaves = the number of leaves per plant in spring. Abiotic cond. = abiotic conditions of the site (wet/dry). The signs show the directions of the significant relationships (+ in No. leaves means more leaves in the spring; + in locality means locality 1, – means locality 2; + in abiotic cond. means wet site, + in year means first year). n.s. indicates non-significant relationships, with $P > 0.05$. Df Error = 3080.

(Table 1), and leaf necrosis, probably a result of previous leaf damage.

Rust fungus *Puccinia punctiformis* occurred in our study significantly more often in plants damaged by folivores, such as *C. rubiginosa*. This observation concurs with the results of Kluth *et al.* (2001), who found that aphids and other beetles preferred plants infected by *P. punctiformis*, even though miners and other oligophagous insects (including *C. rubiginosa*) preferred uninfected *C. arvense*. Tipping (1993) found that herbivory by *C. rubiginosa* was similar between healthy ramets and those infected with rust fungus.

The observed co-occurrence of necrosis at the top of the ramets and flowerhead herbivory probably resulted from damage caused by the same insect species, such as *Larinus planus* and *Rhinocyllus conicus* (Table 1). The importance of these insects for flowerhead damage in *C. arvense* corresponds with the results of Nakamura and Nakamura (2004), who found that three *Larinus* species and three tephritid flies species were the main insects in the flowerheads of *C. arvense*.

Factors determining the occurrence of individual damage types

Plant sizes were estimated in the spring, prior to any herbivore damage, and can thus be considered the cause and not the consequence of the damage. The data indicate that different types of damages tend to occur on plants of different size. These results are in agreement with those of Freese (1993), who suggested that different herbivores tend to select plants in different life stages and different sizes. We can therefore only offer very limited support for the hypothesis from Cebrián and Duarte (1994), i.e. that herbivores prefer larger plants. The results also did not confirm the Vigour Hypothesis (Price, 1991). This is because we demonstrated that *C. arvense* plants experience the same herbivore damage in wet sites and dry sites. Some damage types have stronger effects in wet sites, while others have stronger effects in dry sites, lending only very weak support for the prediction that more stressed plants should suffer greater herbivore damage.

Occurrence of the different damages was affected by year, indicating strong variability in herbivore occurrence between years. Similarly, in a study by Eber and Brandl (2003), *Urophora cardui* was observed on *C. arvense* over 5 years and the infestation rate varied strongly among years. Strong variation in the occurrence of damage was also found among the studied localities. This corresponds to a study from Tipping (1993), who found that the densities of *Cassida rubiginosa* varied among different *C. arvense* populations.

In our study, 84% of *C. arvense* plants were damaged by herbivores. This attack rate was therefore higher than the attack rate (68%) measured for *C. arvense* in Germany (Freese, 1993). However, our value corresponds with the observed attack rate for other *Cirsium* species reported by Freese (1993) and to the results from Skuhrovec *et al.* (2008), who reported over 90% damage on various species of the Carduoideae subfamily. This suggests that the intensity of herbivore damage found in our study is comparable with previous findings for this species.

Effect of natural enemies on the growth of C. arvense

Plants with folivory, stem damage, leaf necrosis and herbivory of the shoot top grew less than those lacking this damage. The first three damage types were also the

Table 5 Effect of natural enemies on basal stem diameter in the autumn and number of flowerheads of *Cirsium arvense*, using the basal stem diameter in the spring as a covariate

	Basal stem diameter		No. of flowerheads	
Damage	Single	Step	Single	Step
Leaf necrosis	0.009 –	n.s.	0.097 –	n.s.
Folivory	0.008 –	n.s.	0.166 –	0.156 –
Stem damage	0.008 –	0.005 –	0.192 –	0.025 –
Herbivory of shoot top	0.008 –	n.s.	n.s.	n.s.
Flowerhead herbivory	n.s.	n.s.	0.13 –	0.008 –
Rust	0.009 –	n.s.	0.097 –	n.s.
Mines	0.009 –	n.s.	0.092 –	n.s.
Leaf necrosis × Year	0.001	0.001	0.022	n.s.
Folivory × Year	n.s.	n.s.	n.s.	n.s.
Stem damage × Year	n.s.	n.s.	n.s.	n.s.
Herbivory of shoot top × Year	0.003	0.003	n.s.	n.s.
Flowerhead herbivory × Year	n.s.	n.s.	n.s.	n.s.
Rust × Year	n.s.	n.s.	0.009	n.s.
Mines × Year	0.0001	n.s.	0.011	n.s.
Leaf necrosis × Abiotic c.	0.004	n.s.	n.s.	n.s.
Folivory × Abiotic c.	0.005	n.s.	0.025	n.s.
Stem damage × Abiotic c.	0.005	n.s.	n.s.	n.s.
Herbivory of shoot top × Abiotic c.	0.004	n.s.	n.s.	n.s.
Flowerhead herbivory × Abiotic c.	n.s.	n.s.	n.s.	n.s.
Rust × Abiotic c.	0.006	n.s.	n.s.	n.s.
Mines × Abiotic c.	0.003	n.s.	n.s.	n.s.
Abiotic conditions		n.s.		n.s.
Locality		n.s.		0.016 –
Year		0.018 –		0.023 –

'Step' means stepwise regression, including all of the plant damage types in a single model, and 'single' is a separate regression for each plant damage type. For a detailed description of the models, see Materials and methods. The values are R^2 values for significant tests ($P \leq 0.05$). The signs show the direction of the relationship, with + meaning positive effect of that damage type, first year, locality 1 and dry site. Df Error = 3085 for single tests and 3067 for stepwise tests.

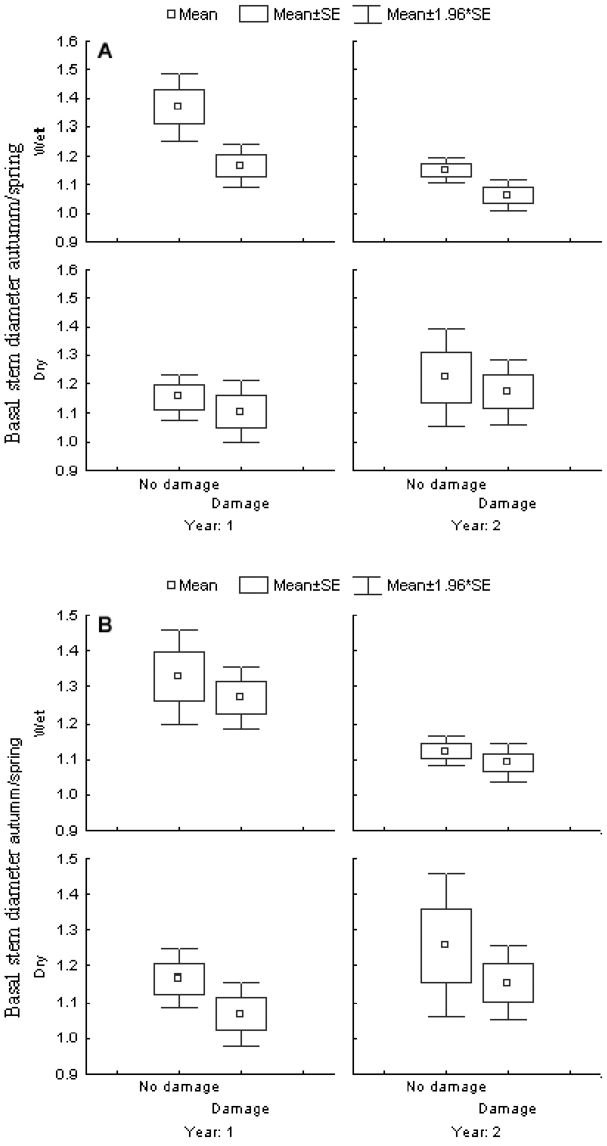

Fig. 2 Effects of (A) stem damage and (B) folivory in different seasons and abiotic conditions of the locality (dry or wet) on plant growth. Plants with or without damage (*x*-axis). The *y*-axis represents the ratio between basal stem diameter in the autumn and the spring.

most common in our study. We can thus assert that the most common plant damage types also had the largest effects on plant growth. When the effects of all damage types were co-analysed, stem damage significantly reduced plant growth, measured as change in basal stem diameter. One cause for this outcome could be that we measured plant growth as changes in the basal stem diameter, and stem damage is thus likely to correlate with this measure of plant growth. For the number of flowerheads, stem damage, folivory and herbivory of the shoot top had significant negative effects on plant reproduction. Despite the fact that the effect of herbivores on plant growth is crucial for understanding their long-term consequences on plant performance, most studies on plant insect herbivores record plant status at only one time point. They can therefore say that plants attacked by a given herbivore are smaller than plants that escaped the attack; however, it is not possible to separate the effects of insects attacking smaller plants, as discussed previously, from the negative effects from insects on plant growth (e.g. Tipping, 1993; Kluth *et al.*, 2001; Eber & Brandl, 2003). Studies that compare *C. arvense* plant size on multiple occasions over a field season include a study from Bacher and Schwab (2000), which demonstrated a negative effect from folivory by *C. rubiginosa* on plant growth. A negative effect from flowerhead insect guilds on *C. arvense* reproduction was

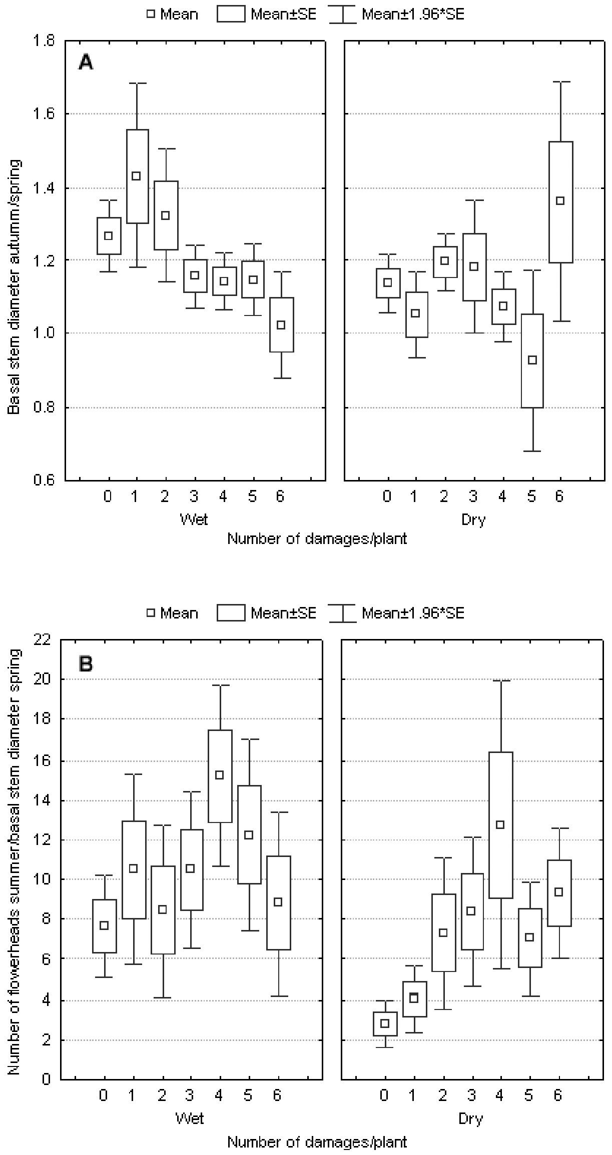

Fig. 3 Effect of the number of damage events per plant on (A) basal stem diameter growth and (B) number of flowerheads per plant in the first and second years. The *y*-axis represents the ratio between (A) basal stem diameter in the autumn and in the spring and (B) number of flowerheads per plant in the summer and basal stem diameter in the spring.

observed by Louda and Potvin (1995) and Skuhrovec *et al.* (2008).

We performed analyses on the effects of single plant damage types on plant growth and reproduction, as this is usually carried out in other studies. However, we also did a stepwise analysis on the effect of all of the plant damage types on plant growth and reproduction. In this way, results from our study can be compared with both one-insect studies and studies that include multiple insects, and we encourage future studies to adopt this method of presenting results.

When multiple herbivores interact with a single plant individual, it is useful to determine whether the effect of the herbivores is additive or complementary (e.g. James *et al.*, 1992; Mcevoy *et al.*, 1993). However, identification of the possible interactions between herbivores and the plants in our study was not possible, because the occurrence of some herbivores was strongly correlated. We did not observe plants suffering damage from only a single herbivore or from that herbivore in combination with each of the other damage types. Such data would require additional experimental manipulations.

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References

Ang BN, Kok LT, Holtzman GI & Wolf DD (1994) Canada thistle (*Cirsium arvense*) response to stimulated insect defoliation and plant competition. *Weed Science* **42,** 403–410.

Bacher S & Schwab F (2000) Effect of herbivore density, timing of attack and plant community on performance of creeping thistle *Cirsium arvense* (L.) Scop. (Asteraceae). *Biocontrol Science and Technology* **10,** 343–352.

Cebrián J & Duarte CM (1994) The dependence of herbivory on growth-rate in natural plant-communities. *Functional Ecology* **8,** 518–525.

Cobb NS, Mopper S, Gehring CA, Caouette M, Christensen KM & Whitham TG (1997) Increased moth herbivory associated with environmental stress of pinyon pine at local and regional levels. *Oecologia* **109,** 389–397.

Coombs EM, Clark JK, Piper GL & Cofrancesco AF Jr. (2004) *Biological Control of Invasive Plants in the United States*. 448. Oregon State University Press, Corvallis, OR, USA.

Delfosse ES (2000) Biological control: important tool for managing invasive species. *Agricultural Research* **48,** 2.

Eber S & Brandl R (2003) Regional patch dynamics of *Cirsium arvense* and possible implications for plant–animal interactions. *Journal of Vegetation Science* **14,** 259–266.

Freese G (1993) The insect complexes associated with the stems of seven thistle species. *Entomologia Generalis* **19,** 191–207.

Friedli J & Bacher S (2001) Direct and indirect effects of a shoot-base boring weevil and plant competition on the performance of creeping thistle, *Cirsium arvense*. *Biological Control* **22,** 219–226.

Hatcher PE, Paul ND, Ayres PG & Whittaker JB (1995) Interactions between *Rumex* spp., herbivores and a rust fungus: the effect of *Uromyces rumicis* infection on leaf nutritional quality. *Functional Ecology* **9,** 97–105.

Hufbauer RA & Root RB (2002) Interactive effects of different types of herbivore damage: *Trirhabda* beetle Larvae and *Philaenus* spittlebugs on Goldenrod (*Solidago altissima*). *The American Midland Naturalist* **147,** 204–213.

James RR, Mcevoy PB & Cox CS (1992) combining the cinnabar moth (*Tyria jacobaeae*) and the ragwort flea beetle (*Longitarsus jacobaeae*) for control of ragwort (*Senecio jacobaea*) – an experimental analysis. *Journal of Applied Ecology* **29,** 589–596.

Kasahara YJ (1982) Japan. In: *Biology and Ecology of Weeds* (eds W Holzner & N Numata), 285–297. W. Junk, The Hague, The Netherlands.

Kluth S, Kruess A & Tscharntke T (2001) Interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects on creeping thistle. *Journal of Applied Ecology* **38,** 548–556.

Louda SM & O'Brien CW (2002) Unexpected ecological effects of distributing the exotic weevil, *Larinus planus* (F.), for the biological control of Canada thistle. *Conservation Biology* **16,** 717–727.

Louda SM & Potvin MA (1995) Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant. *Ecology* **76,** 229–245.

Mcevoy PB, Rudd NT, Cox CS & Huso M (1993) Disturbance, competition, and herbivory effects on ragwort *Senecio jacobaea* populations. *Ecological Monographs* **63,** 55–75.

Moore RJ (1975) Biology of Canadian weed *Cirsium arvense* (L.) Scop. *Canadian Journal of Plant Science* **55,** 1033–1048.

Münzbergová Z (2006) Ploidy level interacts with population size and habitat conditions to determine degree of herbivory damage in plant populations. *Oikos* **115,** 443–452.

Nakamura A & Nakamura K (2004) Faunal make-up, host range and infestation rate of weevils and tephritid flies associated with flower heads of the thistle *Cirsium* (Cardueae: Asteraceae) in Japan. *Entomological Science* **7,** 295–308.

Price PW (1991) The plant vigor hypothesis and herbivore attack. *Oikos* **62,** 244–251.

Rahman A (1982) New Zealand. In: *Biology and Ecology of Weeds* (eds W Holzner & N Numata), 299–308. W. Junk, The Hague, The Netherlands.

Rhoades DF (1985) Offensive–defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. *American Naturalist* **12,** 205–238.

Skuhrovec J, Koprdova S & Mikulka J (2008) How can seed feeders regulate dispersion of thistles, *Cirsium arvense* and *C. heterophyllum*? *Journal of Plant Diseases and Protection* **21,** 281–284.

S-PLUS (2000) *Professional Edition for Windows, Release 2*. MathSoft, Inc., Seattle, WA, USA.

Story JM, Desmetmoens H & Morrill WL (1985) Phytophagous Insects Associated with Canada Thistle, *Cirsium arvense* (L.) Scop, in Southern Montana. *Journal of the Kansas Entomological Society* **58,** 472–478.

Tipping PW (1993) Field studies with *Cassida rubiginosa* (Coleoptera, Chrysomelidae) in Canada thistle. *Environmental Entomology* **22,** 1402–1407.

Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM & Webb DA (eds) (1976) *Flora Europaea. Vol. 4 Plantaginaceae to Compositae (and Rubiaceae)*. Cambridge University Press, Cambridge, UK.

Waring GL & Cobb NS (1992) The impact of plant stress on herbivore population dynamics. In: *Plant-Insect Interactions*, Vol. 4 (ed. EA Bernays), 167–226. CRC, Boca Raton, FL.

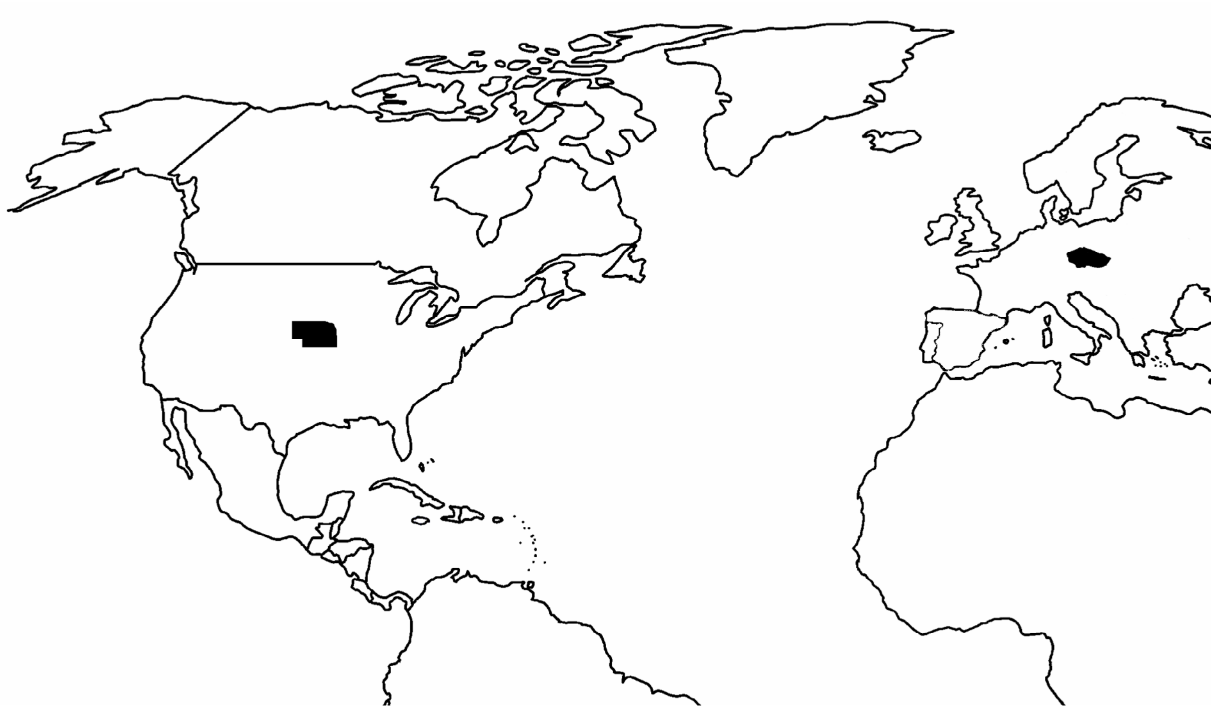
White TCR (1974) A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in New Zealand. *Oecologia* **16,** 279–301.

CHAPTER 2

Degree of damage by natural enemies of *Cirsium arvense* in its native and invasive range

Inés Abela Hofbauerová and Zuzana Münzbergová

Manuscript prepared for submission



Degree of damage by natural enemies of *Cirsium arvense* in its native and invasive range

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Summary

The enemy release hypothesis (ERH) says that plant species in their invasive range will perform better than in their native range due to the release from natural enemies. To test this hypothesis it is appropriate to study a single species in both the native and the introduced range. *Cirsium arvense* observational studies had been done in the native range in the Czech Republic (Europe) and in the invasive range in Nebraska (North America) in natural populations assessing degree of plant damage by insect herbivores.

The results show that *C. arvense* plants are more damaged by a wide range of natural enemies in the native range than in the invasive range. An exception was flower head necrosis, which was not significantly different between ranges. The plants in the invasive range were higher than in the native range and larger plants tended to be more damaged. Higher intensity of damaged was significant in plants from the native range even when taking into account the differences in plant size. The results confirm that in spite of being larger, the plants in the invasive range suffer from less herbivore damage supporting the enemy release hypothesis.

Keywords: *Herbivory, Cirsium arvense, Nebraska, Czech Republic, native and invasive range, plant damage, plant growth, plant reproduction, enemy release hypothesis..*

Introduction

Insect-plant interactions had been lately a focus of many studies and the rapidly growing interest is shown by increasing scientific papers in the last three decades (Schoonhoven et al.2005).With the rising problem of invasive plants, herbivore insects have been studied for use as control agents (Coombs et al. 2004). Many observational studies of plant growth and damage, herbivory experiments and weed control strategy studies have been done on invasive species in its invasive or native range. An example are herbivory experiments (Hinz, Müller-Schärer 2000, Cripps et al. 2010) and weed control strategy studies (Müller-Schärer et al. 2004, Pywell et al. 2010) on *Cirsium arvense* and other species in New Zealand, Great Britain, Austria or Canada. There is still, however, a need for comparison of observational data in both ranges.

The main idea of biological control is that plants in the invasive range escaped their natural enemies and they thus suffer lower herbivore damage. This has been described as the enemy release hypothesis (Keane, Crawley 2002), which has been many times cited but there are not as many plant damage comparative studies which test this hypothesis. Even thou the success of biological control has been used as support for ERH, this evidence does not directly test it (Keane, Crawley 2002).The enemy release hypothesis also states that invasive potential of some non-indigenous species may be enhanced by absence of natural enemies, such as specialist herbivores, in the introduced range (Kolar, Lodge 2001, Keane, Crawley 2002).

Many herbivore insects of *Cirsium arvense* have been studied in an effort to control it, for example the leaf- and bud-feeding beetles [*Altica carduorum* Guerin- Meneville, *Lema cyanella* (L.), and *Cassida rubiginosa* Muller, Coleoptera: Chrysomelidae], stem-boring beetles (*Ceutorhynchus litura* (F.), Coleoptera: Curculionidae), seed-feeding beetles (*Rhinocyllus conicus* (F.), Coleoptera: Curculionidae), stem-galling flies (*Urophora* spp., Diptera: Tephritidae)(Michels et al. 2008) and other insects (Rees 1991; Julien, Griffiths 1998; Campobasso et al. 1999; Gassmann 2005). Even though many studies on insect presence in invasive plants have been done, not many studies look on the level of damage caused by these insects; few data quantify the degree to which naturalized plants are released from natural enemies relative to their native range (Mitchell, Power 2003).

During periods of range expansion, weedy plants may evolve rapidly in adapting to new habitats (Müller-Schärer 2004). Due to the absence of natural enemies, the plants in the invasive range can invest more resources into growth. This was described as the EICA hypothesis (Blossey, Notzold, 1995) which says that plants in the invasive range may grow bigger than plants in the native range. The differences in size can in fact also be one of the factors explaining differences in the degree of damage of the plants.

The aim of this study was to observe naturally occurring plant insect damage of the invasive species *Cirsium arvense* in its native range in the Czech Republic (Europe) and in its invasive range in Nebraska (North America). We wanted to answer the following questions i) What is the intensity of different types of damage on plants from the native and invasive range? ii) Can the differences in the degree of damage be explained by differences in plant size?

Methods and materials

Study species

Cirsium arvense (L.) Scop. (Creeping thistle, California thistle, Canada thistle) is an erect polycarpic perennial herb with horizontal roots, with numerous adventitious non-flowering and flowering shoots, leaves with frequent short strong spines and it is incompletely dioecious (Tiley et al. 2010). It is native to all of Europe, North Africa, Asia Minor, Afghanistan, Siberia, China and Japan (Weber 2005) and invasive in North America, South Africa (Holm et al. 1977), Australia and New Zealand (Rahman 1982).

Study area

Cirsium arvense plant growth and damage were measured in the Czech Republic (Europe) and Nebraska (North America). Nebraska has a continental climate, with highly variable temperatures (Table 1). The Czech Republic has also a continental climate (Table 1) but summers are more moderate than in Nebraska.

Experimental design

In the Czech Republic, Europe (representing the native range of the plant), four populations were studied in June and July 2006 (Table 1) and in total 646 plants were measured.

Eleven populations in total were selected in Nebraska, United States of America, representing the invasive range of *Cirsium arvense*, in June and July 2007 (Table 1). In total 332 plants were measured in Nebraska.

Due to practical reasons more populations were selected in Nebraska with fewer plants and less populations in the Czech Republic with more plants.

Measurements of plant size and plant damage

We took measurements of aboveground herbivore insect damage and plant size. Plant damage measurements included % mines on all the leaves, % plant folivory, % leaf necrosis, number of stem galls per plant, number of cocoons per plant, presence of flower head damage, number of flower heads with necrosis per plant and % external stem damage. Plant measurements were done only once in each plant, in Nebraska in July 2007 and in the Czech Republic in July 2006 (Abela Hofbauerová, Münzbergová 2011). Number of cocoons per plant very rare, therefore this plant damage was not included in the analyses.

Data analyses

To analyze differences in plant damage between the ranges we used general linear models. In the analyses, one of the damages (leaf necrosis, folivory, leaf mines, stem galls, flowerhead damage, flower head necrosis and stem damage) was used as dependent variable. The independent variables were distribution range and population nested within the distribution range. When analyzing flower head necrosis we used number of flower heads as a covariate to take into account that this measure represents number of flower heads and not its proportions. Only flowering plants were included in the dataset when analyzing flower head necrosis and flower head damage.

To see whether the differences in amount of damages per plant could be related to plant size, we also repeated all the above analyses and used plant height and number of flower heads as covariates in the above models.

To see whether the plants differed in size, plant height or number of flower heads were used as dependent variables and distribution range and population nested within the distribution range were used as covariates.

GLM with Poisson distribution was used in all, except for the flower head damage (presence/absence), where binomial distribution was used.

Results

Folivory (Fig. 1), leaf mines, leaf necrosis, stem galls, flower head damage and stem damage were more common in *C. arvense* in the native than in the invasive distribution range. (Table 2).

In all plant damages, except for leaf mines, plant height correlated with plant damages. Higher plants were more damaged with folivory, leaf mines, stem galls, flower head damage, flower head necrosis and stem damages (Fig. 2, Table 2). In contrary, smaller plants suffered higher levels of leaf necrosis (Table 2). The differences in the degree of damage between plants of different size were largely similar when using number of flower heads as a measure of plant size instead of plant height (Table 2). This is due to close correlation between plant height and number of flower heads per plant ($r = 0.54$).

There was a significant interaction between plant height and distribution range in leaf necrosis, stem galls, flower head damage and flower head necrosis. In the native range the relationship between plant height and plant damages was stronger than in the invasive range. A significant interaction between number of flower heads per plant and distribution range was also in folivory, leaf necrosis and stem damage (Table 2). The relationship between number of flower heads and damages was also stronger in the native range.

Plants in the invasive range were higher ($R^2 = 0.02$ ***) but number of flower heads did not significantly differ between ranges. Plant height and number of flower heads varied between populations ($R^2 = 0.217$ *** and 0.191 ***).

Discussion

Our results are in accordance with the ERH which states that invasive plants are released from their natural enemies in the invasive range (Keane, Crawley 2002). Plants were more damaged when they were larger (with exception of folivory, flower head necrosis and leaf mines). In our previous study in the native range we have shown that larger plants are preferred by natural enemies as they are more attractive (Abela Hofbauerová et al. 2011), which is the same as states Cebrián and Duarte (1994) or Price (1991), in the vigour hypothesis. We thus suggest that the difference in the degree of damage of plants of different size is rather the consequence of different size than the cause. Higher attractivity of larger plants is seemingly in contrast with the fact that plants in the native range are smaller and more damaged. By using plant size as a covariate, we, however, demonstrated that the differences between ranges hold even after taking plant size into account. This suggests that higher plants are more attractive for herbivores even when comparing within ranges. In addition, there was a significant interaction between plant height and range for several damages suggesting that the effect of plant size differs between the ranges.

C. arvense were higher in the invasive range, which could be caused by their better performance thanks to natural enemy release. Non-native plants having a better performance is also a statement of the ERH, that has been few times reputed (Colautti et al. 2004), and there are also studies in accordance with it (Torchin et al. 2002, Agrawal, Kotanen 2003, Mitchell, Power 2003, Carpenter, Cappuccino, 2005). Our results are not, however, supporting the ERH in number of flower heads, which is in accordance with Vasquez, Meyer (2011), where the ERH was also partially supported (in a field experiment while it was not supported in the herbivore exclusion experiment). The differences in plant height between the two ranges identified in this study could also be due to different habitat conditions in both areas. However, a garden experiment comparing size of *C. arvense* from both ranges in the same environment suggested that the differences in plants size are likely genetically based (Abela Hofbauerová, Münzbergová 2011)

As a conclusion we showed that naturally occurring *C. arvense* presents less plant damage in the native range than in invasive range and plants in the invasive range are larger than in the native range. This supports the ERH. Our results could also be explained by rapid evolutionary change among populations (Thomson 1998) of *C. arvense*, since during the last five hundred years it is possible the strong genotypes were selected in the invasive range.

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References

Abela Hofbauerová I, Münzbergová Z, Skuhrovec J (2011) The effect of different natural enemies on the performance of *Cirsium arvense* in its native range. *Weed research*, 51(4), 394-403.

Abela Hofbauerová I, Münzbergová Z (2011) Increased performance of Cirsium arvense from the invasive range, Flora (2011), 206 (12) (in press).

Agrawal A.A. & Kotanen P.M. (2003) Herbivores and the success of exotic plants: a phylogenetically controlled experiment. *Ecology Letters*, 6, 712-715.

Cebrián J & Duarte C M (1994) The dependence of herbivory on growth-rate in natural plant-communities. *Functional Ecology* 8, 4, 518-525.

Campobasso G., E. Colonel, L. Knutson, G. Terragitti & M. Cristofaro (1999) Wild plants and their associated insects in the Palearctic Region, primarily Europe and the Middle East. U.S. Department of Agriculture, Agricultural Research Service No. ARS-147. Springfield, Virginia, U.S.A. 243 pp.

Carpenter D. & Cappuccino N. (2005) Herbivory, time since introduction and the invasiveness of exotic plants. *Journal of Ecology*, 93, 315-321.

Colautti R.I., Ricciardi A., Grigorovich I.A. & MacIsaac H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

Coombs M., Clark J K., Piper G. L.& Cofrancesco A.F, Jr. (2004) Biological Control of Invasive Plants in the United States, 448 pages, Oregon State University Press.

Cripps M.G., Edwards G.R., Bourdot G.W., Saville D.J., Hinz H.L. & Fowler S.V. (2010) Effects of pasture competition and specialist herbivory on the performance of Cirsium arvense. *Biocontrol Science and Technology*, 20, 641-656.

Gassmann, A. (2005) Classification of thistle species and potential for biological control of *Cirsium arvense* in New Zealand. Annual Report. CABI Bioscience Switzerland Centre. 25 pp.

Hinz H.L. & Müller-Schärer H. (2000) Influence of host condition on the performance of Rhopalomyia n. sp. (Diptera : Cecidomyiidae), a biological control agent for Tripleutospermum perforatum (Asteraceae). *Biological Control*, 18, 147-156.

Julien M. H. & M. W. Griffiths. (1998) Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds. 4th Edition. CABI Publishing. Oxon, England, UK. 223 pp.

Keane R.M. & Crawley M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164-170.

Kolar C.S. & Lodge D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, 16, 199-204.

Michels G.J., Carney V.A., Lydon J., Ochoa R. & Renn R.L. (2008) New records for *Aceria anthocoptes* (acari: eriophyidae) occurring on canada thistle in colorado, nebraska, and wyoming, usa. *Entomological News*, 119, 483-491.

Mitchell C.E. & Power A.G. (2003) Release of invasive plants from fungal and viral pathogens. *Nature*, 421, 625-627.

Müller-Schärer H., Schaffner U. & Steinger T. (2004) Evolution in invasive plants and implications for biological control. *Trends in Ecology and Evolution*, 19 , 417-422.

Weber E. (2005) Invasive Plant Species of theWorld. CAB International, Wallingford, UK.

Holm L.G., Plucknett D.L., Pancho J.V. & Herberger J.P. eds (1977) *Cirsium arvense* (L.) Scop. The World's Worst Weeds. Distribution and Biology, pp. 217–224. University Press of Hawaii, Honolulu, Hawaii, USA.

Rahman A. (1982) New Zealand. Biology and Ecology of Weeds (eds W.Holzner & N. Numata), pp. 299–308. W. Junk, The Hague, The Netherlands.

Price PW (1991) The plant vigor hypothesis and herbivore attack. *Oikos,* 62**,** 244-251.

Pywell R.F., Hayes M.J., Tallowin J.B., Walker K.J., Meek W.R., Carvell C., Warman L.A. & Bullock J.M. (2010) Minimizing environmental impacts of grassland weed management: can *Cirsium arvense* be controlled without herbicides? *Grass and Forage Science*, 65, 159-174.

Slotta T.A.B., Foley M.E., Chao S.M., Hufbauer R.A. & Horvath D.P. (2010) Assessing Genetic Diversity of Canada Thistle (*Cirsium arvense*) in North America with Microsatellites. *Weed Science*, 58, 387-394L.

Schoonhoven M., J. J. A. van Loon, Dicke M. (2005,) Insect-plant biology, Oxford University Press.

Rees N. E. (1991) Biological control of thistles. pp. 264-273. *In*, L. F. James, J. O. Evans, M. H. Ralphs & R. D. Child (Editors). Noxious Range Weeds. Westview Press, Inc. Boulder, Colorado, U.S.A.
466 pp.

Vasquez E.C. & Meyer G.A. (2011) Relationships among leaf damage, natural enemy release, and abundance in exotic and native prairie plants. *Biological Invasions*, 13, 621-633.

Torchin M.E., Lafferty K.D. & Kuris A.M. (2002) Parasites and marine invasions. *Parasitology*, 124, S137-S151.

Tables and Figures:

Table 1. Information of the studied sites in Nebraska, United States of America, and Czech Republic, Europe.

Population	Altitude(m)	Latitude	Avg daily max temperature (°C)	Avg daily min temperature (°C)	Avg annual precipipation (mm)
Nebraska					
Seward county	425	N 40.82100 W 096.96812	17.2	4.4	714
Jackson west	316	N 41.04391 W 096.62220	16.9	3.7	778
UNL challenge course	368	N 40.85886 W 96.81162	17.2	4.2	728
Jackson 2	312	N 41.04508 W 96.58577	16.9	3.7	778
Cass county North	338	N 40.90007 W 96.38703	17	4.1	749
Platte	853	N 41.09596 W 100.5882	16.9	1.4	507
Lincoln county south west	928	N 40.81609 W 101.11462	17.4	1.3	467
Scottsbluf	1202	N 41,92106 W 103,04804	17.4	1	387
Lyon´s site	1139	N 42.09861 W 102.88511	16.3	0.8	406
Low grass	1158	N 42.31085 W 102.86830	16.3	0.8	406
Sandhills	994	N 42.07976 W 101.43747	15.8	0.6	580
Gosper County	747	N 40.67243 W 99.83813	16.8	3.1	571
Czech Republic					
Kytín	460	N 49º 51' 06" E 14º 12' 37"	12.5	3.4	590
Výžerky	340	N 49° 56' 56" E 14° 53' 95"	13	4.2	590

Table 2. Effect of distribution range, plant height and number of flower heads on percentage of plant damages on *Cirsium arvense*. R^2 values for significant factors are shown, where *** means p-values < 0.0001, ** means p-values < 0.001 and * means p-values < 0.05. N means that the plants are more damaged in the native range, I means the plants are more damaged in the invasive range. + means the more flower head the plant had or higher the plant was, there was more percent of plant damaged in the plant, - means the more flower head the plant had or higher the plant was, there was less percent of plant damaged in the plant.

Plant damage	Distribution range	Plant height	Number of flower heads	Plant height x Distribution range	Number of flower heads x Distribution range
Mines	0.110 *** N	n.s.	0.031 *** +	n.s.	n.s.
Folivory	0.175 *** N	0.002 * +	n.s.	n.s.	0.016 ***
Leaf necrosis	0.059 *** N	0.020 *** -	0.028 *** -	0.003 *	0.054 ***
Stem galls	0.045 *** N	0.064 *** +	0.071 *** +	0.025 ***	n.s.
Flower head damage	0.068 * N	0.064 *** +	0.059 *** +	0.142 ***	n.s.
Flower head necrosis	n.s.	n.s.	n.s.	0.021 *	n.s.
Stem damage	0.213 *** N	0.068 *** +	0.017 *** +	n.s.	0.006 ***

Figure 1. Effect of distribution range on *Cirsium arvense* folivory. For R^2 see values in Table 2.

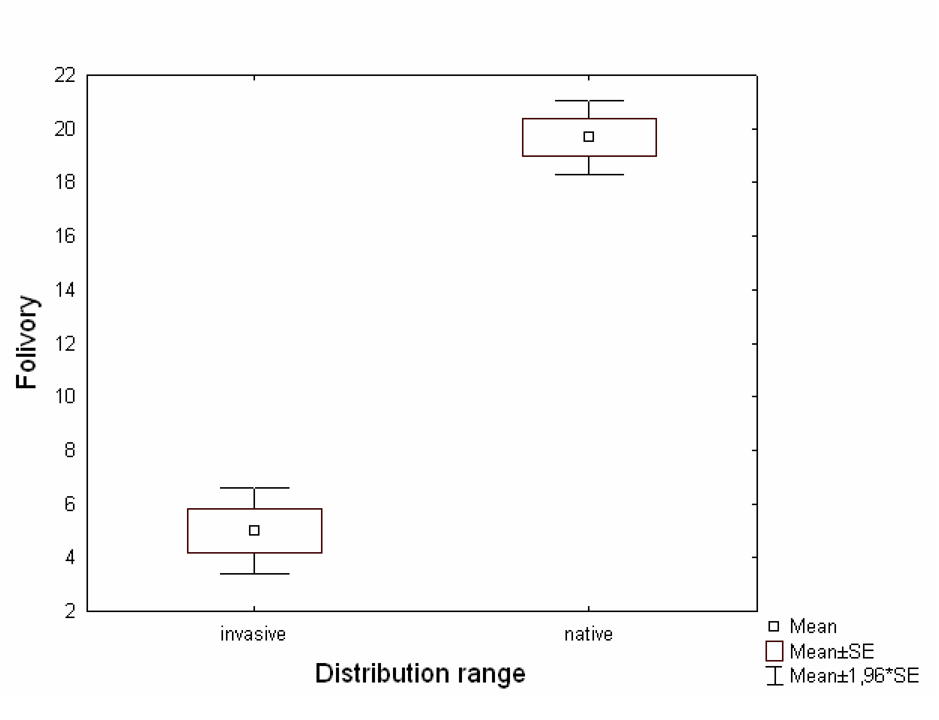


Figure 2. Correlation between plant height and plant damage. For R^2 see values in Table 2.

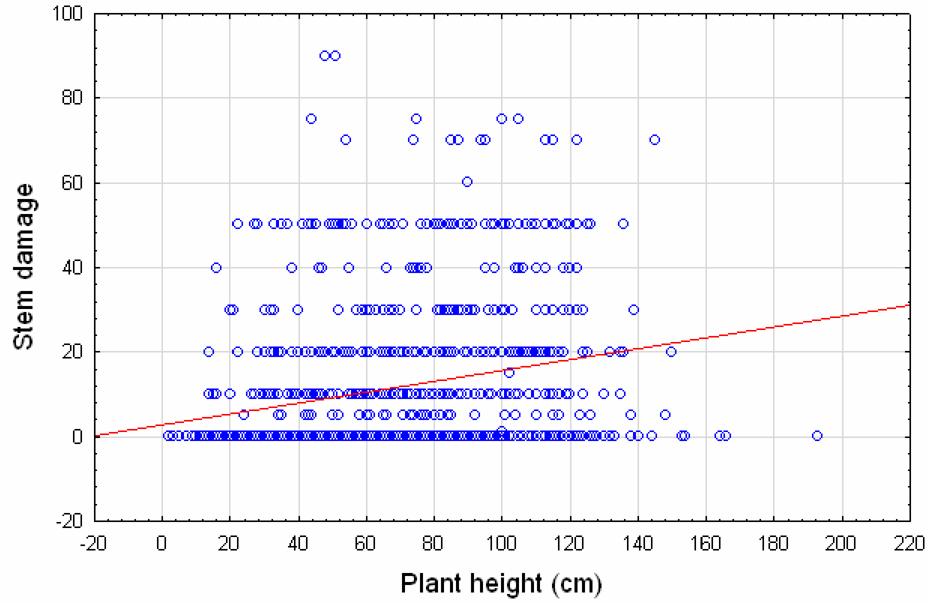
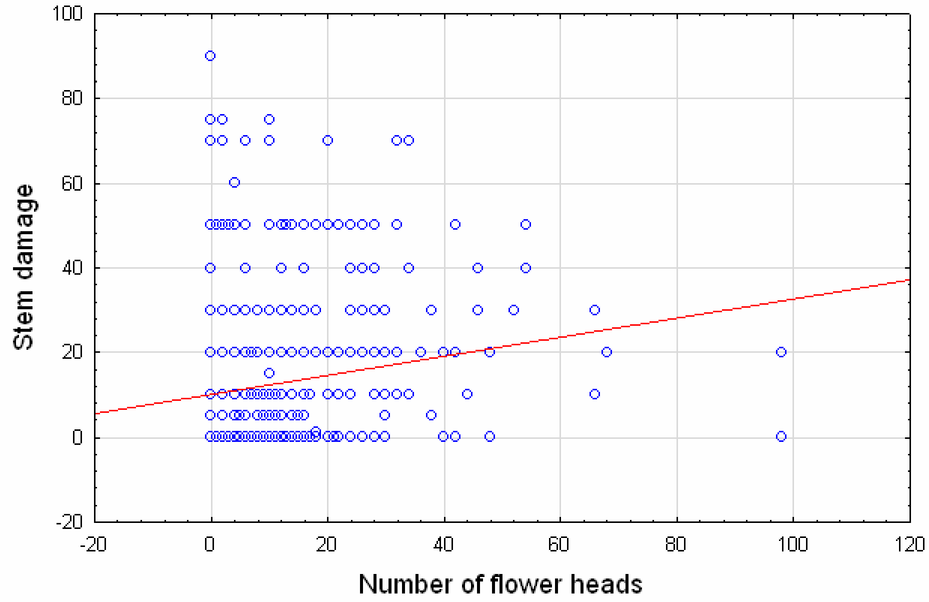


Figure 3. Correlation between number of flower heads and plant damage. For R^2 see values in Table 2.



CHAPTER 3

Effect of aboveground and underground insects on *Cirsium arvense* growth and reproduction from the native and invasive range

Inés Abela Hofbauerová, Zuzana Münzbergová, Leland Russell, Svata Louda and Jiřislav Skuhrovec

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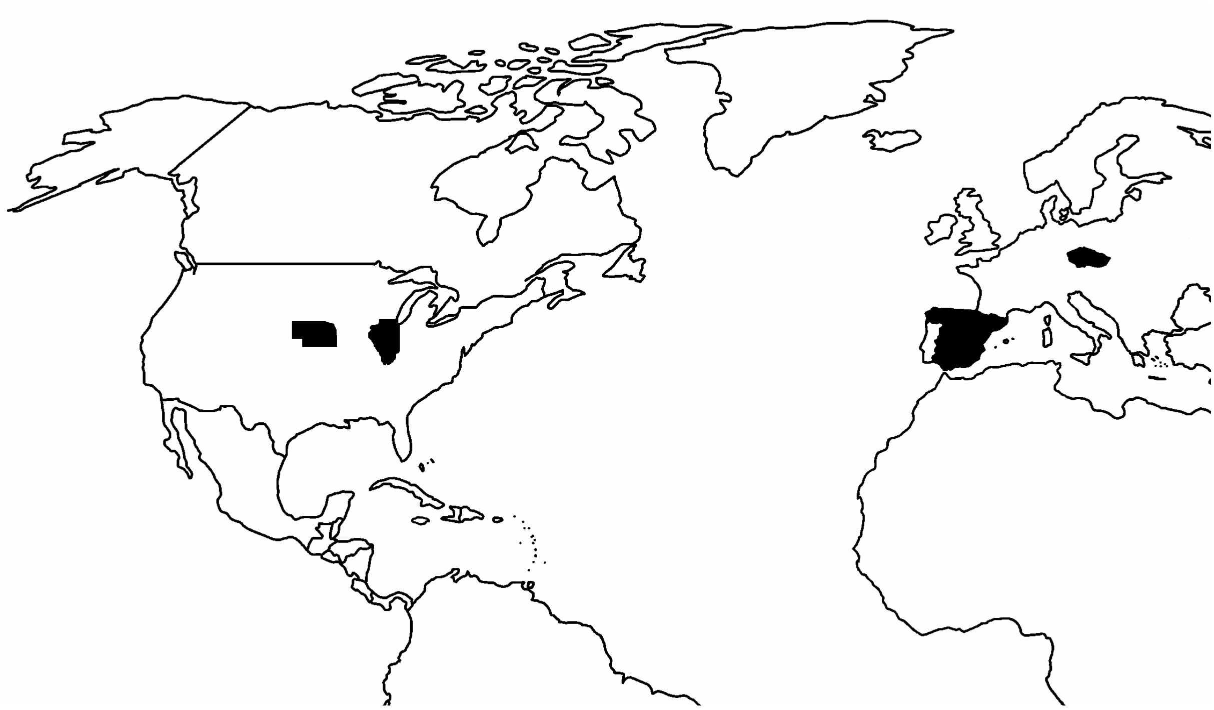

Effect of aboveground and underground insects on *Cirsium arvense* growth and reproduction from the native and invasive range

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Abstract

Testing the most effective herbivore hypothesis and the complementary herbivore hypothesis on a specific insect-plant system can cast light on the mechanisms of herbivory and led to a better choice of biological control agents. The aim of this work is to evaluate the effect of specific natural enemy guilds, represented by specific herbivorous insects and their combinations on *Cirsium arvense* plants from both native and invasive ranges, to test whether these insects can limit growth and reproduction of the invasive species. To do this we performed a common garden experiment in which herbivorous insects were added to *C. arvense* planted in March 2008 and grown from collected seeds from fields in USA (Nebraska, Illinois) and Europe (Spain, Czech Republic). In the aboveground treatments were: added leaf herbivores, added stem herbivores, and added floral/seed head herbivores, , individually and in combination. In a second experiment we stimulated the augmentation of root herbivory. We also manipulated soil resources, by increasing and by reducing soil nutrient availability, in order to examine the effect of soil resources on the effect of herbivores on plant performance.

The expectation, based on the enemy release hypothesis, was that plants from the invasive range would suffer less and be bigger than those from the native range. We also predicted that plants growing in poor soil type would grow less and respond differently to herbivory than plants from the rich soil type.

***Keywords**: Cirsium arvense, Rhinocyllus conicus, Urophora cardui, Cassida rubiginosa, Cleonis pigra, native and invasive range.*

Introduction

Only a few exotic plant species expand and invade natural communities, but those species can pose serious threats to native biodiversity (Myers, Bazely 2003). Understanding the processes that allow some exotic species to experience rapid population growth when introduced to a new geographic region is a critical step in developing strategies to mitigate these species' impacts. The enemy release hypothesis states that invasive potential of some non-indigenous species may be enhanced by absence of natural enemies, such as specialist herbivores, in the introduced range (Kolar, Lodge 2001, Keane, Crawley 2002, Torchin et al. 2002, Mitchell, Power 2003, Colautii et al. 2004, Cappuccino, Carpenter 2005, Vasquez, Meyer 2011). Such specialist herbivores may limit the weed's population growth in its native range (Keane, Crawley 2002). The enemy release hypothesis is the theoretical foundation of classical biological control (Hong et al. 2006), which is based on suppression of pest populations by natural enemies.

There have been many attempts to use biological control to limit spread and density of invasive species (Delfosse 2000) but it is clear that biological control may not only have its benefits but also its threats (Louda et al. 1997, Delfosse 2005). Specifically, several insects introduced for weed biological control have had non-target effects on native plant species. In spite of the extensive existing knowledge on the effects of herbivores on plant population dynamics (Louda et al. 2003), there are some issues that still need to be resolved to implement successful biological control programs. One unresolved issue in biological control concerns whether suppression of weed population growth is maximized using the most important herbivore (most effective herbivore hypothesis) (Turnbull, Chant 1961, Kakehashi et al. 1984, McEvoy et al. 1993) or whether the combined effect of multiple herbivore species is more effective (complementary herbivore hypothesis) (Huffaker, Messenger 1976, Harris 1991, James et al. 1992). Multiple herbivores may not lead to greater suppression of the host plant if, for example, earlier-feeding herbivores alter the plant's chemistry, morphology or phenology in such a way that impact of later-feeding herbivores is reduced. Further, introduction of multiple exotic biocontrol agents, with no increase in efficacy of control, elevates the risk of introducing an agent with non-target effect. Even though both hypotheses have been proposed, their validity has rarely been tested empirically (but see Juenger, Bergelson 1998, Hufbauer, Root 2002).

The few empirical studies of individual vs. joint effects of herbivores on host plants produced mixed results. Juenger and Bergelson (1998) demonstrated that the evolution of flowering phenology in scarlet gilia (*Ipomosis aggregata*) might be a response to diffuse and pair-wise natural selection imposed by multiple herbivores. Hufbauer and Root (2002) found that beetles and spittlebugs feeding together on tall goldenrod (*Solidago altissima*) reduce the mass of the apical bud and the foliage more than would be expected from either insect feeding alone. These studies clearly suggest that the effect of multiple herbivores cannot be easily predicted from the effect of each herbivore separately and further studies on additive and interactive effects of natural enemies on invasive plants are thus sorely needed. Specifically, comprehensive tests of the single and combined effects of herbivore guilds on invasive plants in their native and invasive range are needed to better understand the interaction between natural enemies. Such information may help in designing effective biological control programs. Most of the existing studies on plant-herbivore interactions concentrate only on aboveground herbivores. It was, however, suggested that root feeders in spite of being less diverse than aboveground feeders may have very strong impact on plant population dynamics (Rasman, Agrawal, 2008). It is thus important to consider the impact of root herbivores in pre-release studies for biological control of invasive plants.

The aim of this study is to test the two alternative hypotheses for insect herbivore interactions using *Cirsium arvense* as a model species. *C. arvense* (L.) Scop is one of the most problematic exotic weeds in North America (Moore 1975) and a common native weed in Europe. By measuring damage from various natural enemies on *C. arvense* from native and introduced ranges and analyzing the interactions between the different natural enemies, we tested the most effective herbivore and complementary herbivore hypotheses. We specifically tested whether the effect of single herbivore guilds is independent or if the different herbivores interact with each other. We also wanted to determine whether the effect of herbivores differed between plants from the native and invasive range and between different abiotic conditions.

We asked the following questions: i) Is there any difference in the effect of herbivores on plants grown from seed collected in *C. arvense*'s native vs. invasive range? ii) What are the independent and combined effects of herbivore insects on performance of *C. arvense* and do they differ between ranges?

Methods

Study system

Cirsium arvense (L.) Scop (Carduoideae, Asteraceae) is a herbaceous perennial with erect stems 0.5 - 1.0 m tall, with prickly leaves, and an extensive creeping rootstock (Nuzzo 1997). It is a dioecious plant that also reproduces clonally. It is native to Europe, parts of North Africa and Asia, including Afghanistan, Iran, Pakistan and China. This species was introduced to North America from Europe in the 1600's as a contaminant of grain seed (Jacobs et al. 2006). It is also an invasive species in New Zealand and Australia (Pywell et al. 2010). In USA there is an attempt to manage its invasion by classical biological control (McClay et al. 2001).

In a previous study where plants were not exposed to insect herbivory but were grown in the same common garden as plants for this experiment (Abela, Munzbergova 2011) it was found that plants grown from seed collected in the invasive range had greater aboveground biomass and flower head biomass, more healthy root biomass and longer roots than plants from the native range. Year and type of substrate also affected plant growth, with plants producing more aboveground biomass in the second year but having more flower heads and flower head biomass in the first year, and with plants in the poor substrate having longer roots.

Insect species

Four species of herbivore insects were used to represent the *C. arvense* four main feeding guilds: a leaf beetle, a stem galler, a flower head weevil, and a root-feeding weevil.

The leaf-feeding beetle, *Cassida rubiginosa* (Mueller, 1776) (Coleoptera: Chrysomelidae) is a generalist that feeds on many species of the Carduoideae tribe. It is one of several oligophagous insects on plants of the genera *Carduus* and *Cirsium* and is one of the most conspicuous natural enemies of *C. arvense* (Ang et al. 1995). It is native to Central Europe., and it was intentionally introduced to North America and New Zealand against exotic thistles.

The stem galler, *Urophora cardui* (Linnaeus, 1758) (Diptera: Tephritidae), is a specialist on *C. arvense* (Lalonde, Shorthouse 1985). It attacks stems of Canada thistle, boring in and causing the plant to form terminal stem galls, retarding successful flowering by that stem. Its native distribution extends from France to near the Crimea and from Sweden to the Mediterranean (Rees et al.1996).

Rhinocyllus conicus (Frölich, 1792) (Coleoptera: Curculionidae) is a flower head feeding weevil. It is a generalist that feeds on many species of the Carduoideae tribe. It is native to southern and central Europe, North Africa, and western Asia. This thistle-head weevil was the first insect introduced into North America for the biological control of *Carduus nutanss,* but it is now naturalized and feeding on native thistle species (Louda et al. 1997); its further distribution in USA as biological agent is restricted (USDA 2003, web 1).

Cleonis pigra (Scopoli, 1763) (Curculionidae) is a large, root-feeding weevil. It attacks numerous species of Carduoideae in Europe (Zwölfer 1965). It is native in Eurasia. It was introduced to North America as an agent for biological control of *C. arvense* (L.) (Gassmann et al. 2002), and it now occurs in New York, Pennsylvania, Michigan, Indiana, Ontario, and Quebec.

Seed and insect collection

C. arvense seeds were collected in June, July and August 2007 in Europe and North America, representing the native and invasive ranges of the species (information on sites in Table 1). Using this design we sampled geographically diverse regions of both the native and invasive range with native populations over 2000 km apart and invasive populations over 1200 km apart. The same study sites were used as in our previous study which looked at plant growth without herbivore presence (Abela Hofbauerová, Münzbergová 2011, Table 1).

C. rubiginosa larvae, and later also adults, were collected in the field in the Czech Republic, within 100 km from the experimental garden, in April/May 2008 and 2009, and kept on extra *C. arvense* plants covered with a net which were not part of the experiment. From the insects that reproduced, we obtained larvae for the experiment. Four larvae were added to the leaves of each *C. arvense* plant in the *C. rubiginosa* experimental treatments at the beginning of July 2008 and in June 2009 (see below). Even though *R. conicus* and *U. cardui* are native to Central Europe we were not able to collect sufficient number of adults of these species in the field. We thus ordered insects from a biological control company in Montana, USA. Four *R. conicus* adults (2 males and 2 females) and 4 *U. cardui* adults per plant (2 males and 2 females) were added to the *R. conicus* and *U. cardui* experiment treatments, both individual or in combination treatments, respectively, at the beginning of July 2008 and end of June 2009.We failed to collect *C. pigra* adults in 2008 but in April 2009 sufficient number of insects were collected in the field in the Czech Republic. One *C. pigra* female was added to plants in the *C. pigra* treatments in our experiment at the beginning of May 2009. All insects added to *C. arvense* remained on the plants under a net until autumn, in 2008 and in 2009. The densities of insects added to the plants roughly correspond to the densities of these insects observed in natural populations of the species in the Czech Republic.

Garden experiment

From each distribution range (invasive/native) we used plants from two countries (Europe) or states (USA) and 5 maternal plants per country or state with each plant selected from plants collected at least 100 m apart to reduce the probability they belonged to the same clone. The 5 maternal plants from each country represented replicates in the experiment described below. Performance of control plants (without any insect addition) from this experiment was published elsewhere (Abela and Münzbergová, 2011) and demonstrated that plants from the invasive range are larger that plants from the native range.

To prepare the seeds to be used in the experiment, we dissected the flower heads collected from all natural populations during autumn 2007, and seeds were stored in paper bags. At the end of January 2008, seeds were separated by population in small polyamide marked sacks and then soaked for 10 minutes in 1:10 000 water solution of potassium permanganate to avoid fungal infestation of the growing seedlings. Seeds were placed in pots filled with sand and were covered with a metal net with 1 mm mesh size to avoid insects feeding on seeds. These pots were buried in the ground in an experimental garden for stratification to enhance germination of the seeds in spring. These seeds were extracted from the sand and 5 seeds (in March 2008) were planted in 5 cm x 5 cm pots in a greenhouse without additional heating to germinate. After 3 weeks, we kept one well established seedling in the pot and removed the others. The seedlings were planted in April 2008 in an experimental garden within the native range of *C. arvense*, Czech Republic (N49° 59' 40" E14° 33', 368 m altitude), close to the seed source from the Czech Republic. Plants from the Czech Republic were thus grown in their native conditions, while plants from the other countries were grown in foreign conditions.

C. arvense seedlings were transplanted in large plastic bags, rather than planting directly in the ground, which represented a compromise between ensuring that the large root systems of *C. arvense* had enough room to grow, yet fully controlling the soil environment in which the plants were growing. Two black 100 cm x 75 cm plastic bags, 2 mm thick, were inserted one inside the other. We used two bags to reduce tearing. Bags were filled with 50 l of mixture of substrate using a dredging shovel and 0.5 cm x 0.5 cm holes were cut at the bottom in every bag to avoid excessive soil moisture; to assure drainage, the bottom third of each bag was filled with gravel (1 cm in diameter). To study the effect of soil conditions on plant growth, two types of substrate were used. Half of the plastic bags were filled with soil from the garden nearby the common garden experiments (rich substrate), the other half with sand from a quarry 100 km away from the experimental site (poor substrate). The nutrient rich substrate had significantly higher content of nitrogen, phosphorus, total carbon, carbon in carbonates and carbon in organic matter (Table 2). Due to the high volume of substrate, not all substrate could be sterilized. To limit growth of weeds in the bags, a 6 cm layer of a mixture of 10:1 sterilized sand and perlite was placed on the top of the soil.

Immediately after planting the seedlings, cylindrical metal scaffolds, 39 cm in diameter and 98 cm high, were placed into the bags to cover the plants, and white translucent 108 cm x 72 cm polyamide sacks 28 g/m^2 dense were fastened over each metal scaffold to exclude natural enemies other than those introduced experimentally attacked the plants.

Plant growth and plant damage were measured within two days in August at the time of full flowering in two years (2008, 2009). Plant measurements included: plant height, number of stems, plant sex (when flowering) and number of flower heads. Plant damage included percent of leaves damaged by *C. rubiginosa*, numbers of seeds and of flower heads attacked by *R. conicus*, number of *C. pigra* larvae in root, length of weevil-damaged roots, and the number, weight and dimensions of *U. cardui* galls. Each year after measurements, all aboveground biomass was harvested, dried to a constant weight and weighted. All developed seeds per plant were counted in both years. Because the species is dioecious and about half of the plants were male, it was not possible to evaluate seed production of all the plants. Number of flower heads/plant can be considered as a fitness measure since it strongly positively correlates with number of seeds/plant in the female plants ($R^2 = 0.5$).

During May 2010, the whole experiment was harvested. Roots were carefully extracted from the soil and rinsed to remove all the substrate. Roots were categorized as “healthy,” “damaged” or “dead.” Healthy roots were white. Roots were classified as damaged if they were brown or the root had other clear signs of damage. Roots were considered as dead when they were dark brown and decomposing. Length of healthy parts of the roots wider than 1 mm was measured. Healthy, damaged and dead biomass of the roots were weighed before and after drying to a constant weight.

To describe chemical composition of the two soil substrates, 10 samples from each substrate type were taken (one sample per bag) in May 2010. Content of nitrogen, total carbon, carbon in carbonates, organic carbon and phosphorus was measured in the laboratory of the Institute of Botany, Academy of Sciences of the Czech Republic (Table 1).

Experimental treatments

Using the experimental set-up described above we conducted two herbivore addition experiments. The first, which addressed individual vs. joined effect of aboveground insects, involved 8 treatments. Three treatments contained a single insect species: *C. rubiginosa, R. conicus* or *U. cardui.* Three treatments contained two aboveground insect herbivore species: *C. rubiginosa* and *U. cardui, C. rubiginosa* and *R. conicus, U. cardui* and *R. conicus*. One treatment included all the three aboveground insect species (*C. rubiginosa, R. conicus* and *U. cardui*) and the last treatment was control without insect addition. The second addressed individual vs. joint effects of above- and belowground herbivores. It included 4 treatments. Control plants and plants with all the three aboveground insects corresponded to the treatment in the first experiment. It further contained plants with all aboveground insects and the underground insect *C. pigra* and one treatment with a single insect species, *C. pigra.*

Thus, with plants from two distributional ranges (native, invasive), two countries or states within each distributional range (Europe: Spain, Czech Republic; USA: Nebraska, Illinois), two soil substrates (loam, sand), and five replicates for each of the 10 insect-addition treatments, the total number of experimental plants was 400.

Data analysis

We first analyzed the effect of the addition of aboveground insects using generalized linear models. As dependent variables we used plant height, aboveground biomass, flower head biomass and number of flower heads per plant. Independent variables were type of soil substrate, year of measurement (2008, 2009), seed country/state origin nested within the seed origin range (Spain, Czech Republic, Nebraska, Illinois), seed range origin (invasive and native range of *C. arvense*) and insect (*C. rubiginosa*, *R. conicus* and *U. cardui*) addition treatment. We also included interactions between the independent variables into the model. All the dependent variables followed a Gamma distribution. When analyzing flower head biomass, we performed additional analyses with aboveground biomass as a size covariate, to evaluate the investment of the plants to reproduction.

The effect of addition of both aboveground and underground insects on the same dependent variables was then calculated, using the same methods as in the previous analyses. Independent variables were type of soil substrate, year of measurement, seed country origin, seed range origin and presence of all aboveground insects (*C. rubiginosa*, *R. conicus* and *U. cardui* together yes/no) and underground insects (*C. pigra*). In these analyses, treatment of plants with the addition of one or two aboveground insects was not included. All analyses were done in SPlus 2000 and Statistica 10.

Effect of insect addition on root biomass was analyzed for 2009, since we have data on root biomass only from the end of the experiment. We analyzed the effect of the addition of aboveground and underground insects using generalized linear models. As dependent variables we used healthy root biomass, damaged root biomass, dead root biomass, root length, root : shoot ratio and total plant biomass. Independent variables were type of soil substrate, seed country/state origin nested within the seed origin range (Spain, Czech Republic, Nebraska, Illinois), seed range origin (invasive and native range of *C. arvense*) and insect addition treatment (no addition, aboveground insect addition: *C. rubiginosa*, *R. conicus*, *U. cardui*, only underground *C. pigra* addition, above and underground insects addition).

To analyze whether addition of each herbivore insect alone has effect on plant performance, we compared performance of a plant with addition of a single insect species to performance of a plant originating from the same mother plant without any insect addition. We compared these two groups of plants using Wilcoxon matched pairs test in Statistica 10. To express the strength of this effect we calculated the proportional change in size due to herbivory h as:

$$P_h = (S_c-S_h)/S_c$$

where S_c is size of control plant and S_h is size of the corresponding plant with herbivore h. Positive value indicates that control plant is larger than plant with the herbivore and the herbivore thus reduces plant growth. We did this calculation for each of the 4 herbivore species. In addition, we did this calculation for all the three aboveground insects together (i.e. S_h was size of a plant with all the three aboveground insects together). This information was used for comparing with the underground herbivore below.

To analyze if adding multiple herbivores had higher or lower effect than would be expected from addition of each herbivore separately we defined expected effect of herbivores h1 and h2 as:

$$EP_{h12} = P_{h1} + P_{h2}$$

We compared this expected value with observed effect of herbivores h1 and h2 in combination. This was defined as

$$OP_{h12} = (S_c-S_{h12})/S_c$$

The observed and expected values EP_{h12} and OP_{h1} were compared using Wilcoxon matched pairs test in Statistica 10.

To express to what degree the effect of combining multiple insects differs from the observed effect we calculated strength of the effect as

$PC_{h12} = (OP_{h12} - EP_{h12}) / EP_{h12}$.

Positive PC (proportional effect of combination) value indicates that combination of multiple insects on a single plant has stronger effect than would be expected based on simple additive effect. This calculation was done for all pairs of the three aboveground insects as well as for combination of all the three aboveground insects together. It was also done for combination of the belowground herbivore with the three aboveground insects.

Results

Individual species addition

Main effect of none of the insects on any dependent variable was significant. In contrast, there were many significant interactions between insect addition and year and insect addition and distribution range indicating that the effects of herbivores strongly varies between ranges and between years.

Strong variation between years was visible in the effect of *C. rubiginosa* on flower head biomass per plant (Table 3) and number of flower heads (Fig 1, Table 3). *C. rubiginosa* had a significant negative effect on flower head biomass and number of flower heads in plants from the invasive range and in poor substrate in 2008 compared to control plants, but there was no significant effect in 2009 (Appendix 2 A). The effect of *C. rubiginosa* on flower head biomass and number of flower heads was significant in the whole dataset in 2008 but not in 2009, and was also significant on healthy root biomass in plants from the native range in the rich substrate (Appendix 2 L). The effect of *C. rubiginosa* on plant height and aboveground biomass was not significant in any case.

Similarly to *C. rubiginosa* also the effect of *R. conicus* on flower head biomass interacted with year and seed source distributional range (Table 3). Plants grown from seed collected in the native range with addition of *R. conicus* had more flower head biomass than plants with no *R. conicus* addition in the first year. However, in the second year, flower head biomass dropped dramatically (Fig. 2) and plants from the native range had lower flower head biomass with *R. conicus* added than did plants from the introduced range. Individual addition of *R. conicus* had a significant negative effect on height in plants from the invasive range and poor substrate, in 2008 (Appendix 2 B). In the next year it had a negative effect on plant height in both ranges and substrates (all groups, Appendix 2 B). *R. conicus* had a significant negative effect on aboveground biomass in plants from the invasive range and rich substrate, in 2008 (Appendix 2 B) and on root:shoot ratio in plants from the native range in poor substrate (Appendix 2 M). It had, however, no effect on flower head biomass and number of flower heads.

The effect of *U. cardui* on flower head biomass strongly varied between years and distribution ranges (Table 3). Individual addition of *U. cardui* in 2008 had a significant negative effect on plant height, aboveground biomass, flower head biomass and number of flower heads, comparing to control plants except for plants from the native range in the rich substrate, where the gall former insect had positive effect on plants (Appendix 2 C). The effect was also significantly positive on aboveground biomass in plants from the invasive range in the poor substrate and in the native range in the rich substrate in 2008 (Appendix 2 C). In 2009 the gall former had a negative effect on plant height in the invasive range and poor substrate (Appendix 2 C) but effect in other parameters was not significant in any other case. *U. cardui* did not have any effect on any root growth parameter.

Individual addition of the underground herbivore *C. pigra* did not have any effect on aboveground biomass, plant reproduction, root biomass and root length (Table 5) but it had a significant negative effect on plant height in plants from the invasive and native range and rich substrate (also in all groups) (Appendix 2 D).

Multiple species addition

In plants with addition of all aboveground insects, aboveground biomass, number of flower heads and flower head biomass (Fig.4) were higher (Table 3), but this difference was larger in 2008 than in 2009 and larger in the native range than in the invasive. Flower head biomass and number of flower heads were not significantly different in combination of all aboveground insects than in single insect additions.

Height was affected by the combination of all three aboveground insects in 2009, having plants with all three aboveground insect addition shorter shoots (Fig.5, Table 4). Triple combination of the aboveground insects was stronger than expected in 2009 for plant height and in 2008 for aboveground biomass (Appendix 2 E).

Combination of *C. rubiginosa* and *R. conicus* addition in interaction with substrate had a negative effect on flower head biomass and number of flower heads (Table 3), decreasing in the poor soil substrate in both years. When comparing effect of *C. rubiginosa* and *R. conicus* added in combination to the expected effect from their individual addition, the combination had stronger effect on plant height and aboveground biomass in plants from the invasive range in the poor substrate. The effect was significantly weaker than could be expected from the single insects in plants from the invasive range in the rich substrate in 2008 (Appendix 2 H). In the overall tests, *C. rubiginosa* and *R. conicus* in combination had stronger effects than expected from their separate effect only on plant height in 2009. The effect of addition of this insect combination on root growth parameters and total plant biomass did not significantly differ from the addition of the two insects added separately.

The *C. rubiginosa* and *U. cardui* combined addition negatively affected plant height and aboveground biomass in plants from the invasive range in the poor substrate in 2008 (Appendix 2 I), the effect on plant height and aboveground biomass was negative in plants from the native range in the rich substrate in 2009 (Appendix 2 I). The effect of *C. rubiginosa* and *U. cardui* in 2009 on healthy root biomass and root:shoot ratio in plants from the native range in rich substrate and on root length in plants from the native range in poor substrate was stronger than the expected of both insects when added separately (Appendix 2 N) The overall effects of their combined addition were, however, never significant in aboveground growth parameters, but were significant for healthy root biomass and root length (Appendix 2 N). The *C. rubiginosa* and *U. cardui* was not significantly different than single addition in flower head biomass nor number of flower heads (Appendix 2 I). This indicates that in most cases their combined effect corresponds to effect expected from their single addition.

When adding the combination of *R. conicus* and *U. cardui*, flower head biomass decreased in comparison with control plants but in the native range in the rich substrate flower head biomass increased in plants with this combination of insects (Table 3, Fig.4). Comparing with expected values from single insect addition, combined addition of *R. conicus* and *U. cardui* had stronger effect than expected on aboveground biomass in the invasive range and poor substrate in 2008 and in the native range in the rich substrate in 2009 (Appendix 2 J), but did not differ from single insect addition in plant height, flower head biomass, number of flower heads and none of the root growth parameters.

In the combination of all the aboveground insects with the underground *C. pigra*, the effect was stronger than expected only for plant height in 2009 (Fig. 5, Appendix 2 G). When tested for each treatment separately, the effect was stronger than expected for plant height in rich substrate for native plants in 2009 and for aboveground biomass for invasive plants in poor substrate in 2009 (Fig. 5, Appendix 2 G).

Addition of all aboveground insects in interaction with type of substrate had a positive effect on root:shoot ratio (Table 5), with plants growing from seeds from the invasive range and in poor substrate having higher healthy root biomass. Addition of all aboveground insects had an overall negative effect for plant height. This combination had also a more significant negative effect than expected on healthy root biomass, damaged root biomass and root length in plants from the invasive range in poor substrate type and in all groups, except for healthy root biomass (Appendix 2 K); also had a more negative effect on root:shoot ratio in native range and poor substrate plants than expected and in all groups(Appendix 2K).

Total plant biomass was negatively affected by all aboveground insect addition (Table 5), insect addition reducing total plant biomass. Total plant biomass was also affected by distribution range, substrate, country, and country in interaction with *C. pigra* addition (Table 5) having plants in the invasive range and in rich substrate more total biomass. Combination of all the aboveground insects + *C. pigra* did not have any effect on any root growth parameter nor total plant biomass. In contrary, the overall effects of the combined all aboveground insect addition were more negative than the expected.

Discussion

Effects individual herbivores

The insects used in this study have been promoted as biocontrol agents based on encouraging results in earlier experimental situations. For example, *U. cardui* caused stunted growth and reduced flowering of thistle *C. arvense* (Peschken, Harris 1975, Forsyth, Watson 1985). Also, *R. conicus* attacks on musk thistle (*Carduus nutans* L.) appear to be successful in reducing weed infestations in many locations (Gassmann, Kok 2002), but not necessarily (Louda 1998).

Bacher (2000) found that *C. rubiginosa* has a negative effect on shoot growth in native *C. arvense*. In our study, we surprisingly found a negative effect of folivore *C. rubiginosa* on flower head biomass and number of flower heads in plants from both ranges in 2008 but not on total biomass and plant height. Our results on negative effect of the folivor on root biomass contrast with those from another experimental study in the invasive range (Cripps et al. 2010) where *C. rubiginosa* folivory had a positive effect on *C. arvense* root biomass, but no effect on other growth parameters.

It has been demonstrated in many studies under controlled conditions that the gallfly *U. cardui* has profound negative effects on life history traits of its host *C. arvense* (Sole 2007). Therefore, it is not surprising the result in this study that *U. cardui* reduced *C. arvense* growth and reproduction, even though there was no impact on root growth. It is contrasting, though, with results in the invasive range (Reet et al. 2006) where *U. cardui* did not have any impact on plant height and flower head number, declaring effect of this insect on *C. arvense* growth in the invasive range as not so important.

There are many studies on effect of *R. conicus* on native North American thistles (Arnett, Louda 2002) or European *Carduus nutans* (Jongejans 2006), however, not so much is studied about the effect of *R. conicus* on *C. arvense* in its native range. Even it is reported *R. conicus* did not reduce North American *C. arvense* growth (Arnett, Louda 2002), significant negative effect on plant growth of the flower head weevil was found in our study, confirming the result of non-impact in invasive range also in its native range.

Our results on *C. pigra* effect contrast to those of Forsyth (1983 and references within), where *C. arvense* with *C. pigra* had higher aboveground biomass in the invasive range that the control plants. We did not find a positive effect of *C. pigra* on aboveground biomass but rather a negative effect on plant height in plants grown from invasive and native seeds in the native range. Addition of *C. pigra* did not, however, reduce or increase root biomass nor root length. This is consistent with the result of Ang et al. (1995) suggesting that no root feeders can cause substantial damage to *C. arvense* biomass. Even Schadler (2004) found *C. pigra* damage in *C. arvense* roots in Germany but the effect on root growth was not analyzed. Andrzejewska et al. (2006) found in Poland *C. pigra* negatively impacts *Silybum marianum* crop yield, but this difference in results is probably caused by *C. arvense* big clonal growth and root system.

Most effective herbivore hypothesis vs. the complementary herbivore hypothesis

James et al. (1992), Hufbauer and Root (2002) and Juenger and Bergelson (1998) supported the complementary herbivore hypothesis by showing that combined effects of paired insects reduce growth of *Senecio jacobea*, *Solidago altissima* and *Ipomopsis* agg. more than when added separately. In this present study, paired combinations *C. rubiginosa* + *R. conicus*, *C. rubiginosa*+*U. cardui* and *R. conicus*+*U. cardui* have been tested on *C. arvense* and larger effect on the plant was found for double insect addition than would be expected based on the single insect addition. This was, however, true only for some treatments in some years.

Although above and underground plant organs are interconnected, most research studying plant-insect interactions focused either on root herbivory or on shoot herbivory in isolation (Bezemet et al. 2003). Root herbivory has been reported to reduce foliar feeding insect performance (Gange 2001). In our study we used both a root herbivore and representatives of the aboveground herbivore guilds (foliar leaf beetle, floral flower head weevil, gall forming stem-feeder). Therefore, in addition to quantifying the effect of foliar, floral and stem herbivory, we found that the above and belowground insects combined reduced plant height (native range) and aboveground biomass (invasive range) in certain years and substrates, but did not reduce root biomass (with the exception of *C. rubiginosa* and *U. cardui* combination, which reduced some root parameters in the native range), showing that *C. arvense* clonal root growth can not be easily controlled. So, even it is thought that root feeders can have a large impact on plant roots (Rasman, Agrawal 2008) we did not find any effect on root growth.

The results in this study partially support the complementary herbivore hypothesis since effect of addition of insects was bigger than when added separately. The effect was, however, not always significant suggesting that the insects are complementary under some circumstances but not under other.

Conclusion

The study showed that all the four tested insect species can reduce growth of *C. arvense* when added separately. Effect of the insects was significant only in some years and for some response variables. Surprisingly, the leaf feeding *C. rubiginosa* affected mainly flower head biomass and number of flower heads but had no effect on plant height and aboveground biomass. In contrast, the flower head feeding *R. conicus* had effect mainly on plant height and aboveground biomass. The gall forming *U. cardui* affected all the size measures. The root boring *C. pigra* significantly affected only plant height. All this indicates that the different insect species have a differential effect on various measures of plant performance. In addition, the different insects had effects in different years, in different substrates and for plants from different ranges. All this suggests that a combination of multiple herbivores could be a better approach for controlling the plant than adding a single insect species as the insects seem to be largely complementary. This complementarity between the insects was observed for all the combinations. The effect of multiple insects on a single plant was generally stronger than expected from their simple addition. This effect was, however, significant only in some years, for some response variables, for some substrates and some plant origins. In a few cases, combination of the insects had significantly weaker effect than expected.

All the studied insects had been used as biological control on *C. arvense* but never had strong large scale impact on reducing populations in North America and New Zealand (Jacobs 2006, Cripps et al. 2011). Even though we found negative effect of insect addition on *C. arvense* growth and reproduction in some substrate types and distribution ranges, we suggest being cautious on release of *C. rubigonosa*, *U. cardui*, *R. conicus* and suggest that *C. pigra* will not be a successful control agent.

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References

Anderson, R. S. (1987). Systematics, phylogeny and biogeography of New World weevils of the tribe Cleonini (Coleoptera: Curculionidae) *Quaestiones Entomologicae* 23: 431-709.

S-PLUS (2000) Professional Edition for Windows, Release 2. MathSoft, Inc.

Statistica 10, Statsoft.

Andrzejewska J; Lamparski R; Skinder Z (2006). Assessing the extent of damage caused by *Cleonus piger* Scop. and other entomofauna in the cultivation of milk thistle [*Silybum marianum* (L.) Gaertn.]. *Journal Of Plant Protection Research*, 46, 49-60.

Ang, B.N., Kok, L.T., Holtzman, G.I. & Wolf, D.D. (1995) Canada thistle (*Cirsium arvense*) (L.) Scop.) response to density of *Cassida rubiginosa* Müller (Coleoptera:Chrysomelidae) and plant competition. *Biological Control*, 5, 31–38.

Bacher S. & Schwab F. (2000) Effect of herbivore density, timing of attack and plant community on performance of creeping thistle *Cirsium arvense* (L.) Scop. (Asteraceae). *Biocontrol Science and Technology*, 10, 343-352.

Blossey B. ,Notzold R. (1995) Evolution Of Increased Competitive Ability In Invasive Nonindigenous Plants - A Hypothesis. *Journal of Ecology*, 83, 887-889.

Assessment, O. O. T. (1995) Biologically Based Technologies for Pest Control. OTA Publications OTA-ENV-636.

Bezemer T.M., Wagenaar R., Van Dam N.M. & Wackers F.L. (2003) Interactions between above- and belowground insect herbivores as mediated by the plant defense system. *Oikos*, 101, 555-562.

Cappuccino, N. & Carpenter, D. (2005) Invasive exotic plants suffer less herbivory than non-invasive exotic plants. *Biology Letters*, 1, 435-438.

Colautti R.I., Ricciardi A., Grigorovich I.A. & MacIsaac H.J. (2004) Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, 7, 721-733.

Crawley, M. (1989) The successes and failures of weed biocontrol using insects. *Biocontrol News and Information*, 10, 213–223.

Delfosse, E. S. (2000) Biological control: important tool for managing invasive species. *Agricultural Research*, 48, p. 2.

Delfosse, E. S. (2005). Risk and ethics in biological control. Biological Control 35:319-329.

Eber S., Brandl R. (2003) Regional patch dynamics of *Cirsium arvense* and possible implications for plant-animal interactions. *Journal of Vegetation Science*, 14, 259-266.

Fenner M., Lee W.G. (2001) Lack of pre-dispersal seed predators in introduced Asteraceae in New Zealand. *New Zealand Journal of Ecology*, 25, 95-99.

Friedli J, Bacher. S. (2001) Mutualistic interaction between a weevil and a rust fungus, two parasites of the weed *Cirsium arvense Oecologia* 129, 571–576.

Gange A.C. (2001) Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist*, 150, 611-618.

Gassmann A., Tosevski I., Diefer B., Schneider H. (2002) Biological Control of Canada Thistle, *Cirsium arvense*. CABI Bioscience Switzerland Centre, Annual Report for 2002, p. 18.

Gassmann, A., L.T. Kok. 2002. Musk Thistle (Nodding Thistle). Pp. 229–245. *In* R. van Driesche, B. Blossey, M. Hoddle, S. Lyon, and R. Reardon (Eds.). Biological Control of Invasive Plants in the Eastern United States. USDA Forest Service, Morgantown, WV. 413 pp.

Harris P. (1991) Invitation paper (Alexander,c.p. Fund) - Classical Biocontrol Of Weeds - Its Definition, Selection Of Effective Agents, And Administrative Political Problems. Canadian Entomologist, 123, 827-849.

Hong Liu, P. S., R. W. Pemberton, J. Peña. (2006) Insect Herbivore Faunal Diversity Among Invasive, Non-Invasive And Native Eugenia Species: Implications For The Enemy Release Hypothesis. *Florida Entomologist* 89, 475.

Hufbauer R. A., Root R. B. (2002) Interactive Effects of Different Types of Herbivore Damage: *Trirhabda* beetle Larvae and *Philaenus* spittlebugs on Goldenrod (*Solidago altissima*). *The American Midland Naturalist*, 147, 204–213.

Huffaker, P. Messemger (1976) Theory and practice of biological control. book.

Cofrancesco, A. F. (1998) Overview and future direction of biological control technology. *Journal of Aquatic Plant Management*, 36, 49-53.

Jacobs J, S. J., Menalled F (2006) Ecology and Management of Canada thistle [*Cirsium arvense* (L.) Scop.]. Montana Invasive Species Technical Notes, No. 5.

James R.R., McEvoy P.B. & Cox C.S. (1992) Combining The Cinnabar Moth (*Tyria-Jacobaeae*) And The Ragwort Flea Beetle (*Longitarsus-Jacobaeae*) For Control Of Ragwort (*Senecio-Jacobaea*) - An Experimental-Analysis. *Journal of Applied Ecology*, 29, 589-596.

Jongejans E., Sheppard A.W. , Shea K. (2006) What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range? *Journal of Applied Ecology*, 43, 877-886.

Juenger T., Bergelson J. (1998) Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. *Evolution*, 52, 1583-1592.

Kakehashi N., Suzuki Y. & Iwasa Y. (1984) Niche Overlap Of Parasitoids In Host Parasitoid Systems - Its Consequence To Single Versus Multiple Introduction Controversy In Biological-Control. *Journal of Applied Ecology*, 21, 115-131.

Keane R.M. & Crawley M.J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution*, 17, 164-170.

Kluth S., Kruess A. & Tscharntke T. (2001) Interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects on creeping thistle. *Journal of Applied Ecology*, 38, 548-556.

Kluth S., Kruess A. & Tscharntke T. (2002) Insects as vectors of plant pathogens: mutualistic and antagonistic interactions. *Oecologia*, 133, 193-199.

Kluth S., Kruess A. & Tscharntke T. (2003) Influence of mechanical cutting and pathogen application on the performance and nutrient storage of *Cirsium arvense*. *Journal of Applied Ecology*, 40, 334-343.

Kok, L. T. and Surles, W. W. (1975). Successful biocontrol of musk thistle by an introduced weevil, *Rhinocyllus conicus*. *Enviroomental. Entomology.* 4:1025-1027.

Kolar C.S. & Lodge D.M. (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution*, 16, 199-204.

Kruess A. (2002) Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*. *Oecologia*, 130, 563-569.

Lalonde R.G. & Shorthouse J.D. (1985) Growth And Development Of Larvae And Galls Of *Urophora-Cardui* (Diptera, Tephritidae) On *Cirsium-Arvense* (Compositae). *Oecologia*, 65, 161-165.

Louda S.M. (1998) Population growth of *Rhinocyllus conicus* (Coleoptera : Curculionidae) on two species of native thistles in Prairie. *Environmental Entomology*, 27, 834-841.

Louda S.M. & Potvin M.A. (1995) Effect Of Inflorescence-Feeding Insects On The Demography And Lifetime Fitness Of A Native Plant. *Ecology*, 76, 229-245.

Louda S.M., Kendall D., Connor J. & Simberloff D. (1997) Ecological effects of an insect introduced for the biological control of weeds. *Science*, 277, 1088-1090.

Louda S.M., Pemberton R.W., Johnson M.T. & Follett P.A. (2003) Nontarget effects - The Achilles' Heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. *Annual Review of Entomology*, 48, 365-396.

Lund, S. & Rostrup, E. (1901) Marktidselen, *Cirsium arvense*. En monografi.Dem Kongelige Danske Videnskabernes Selskabs Skrifter, Raekke 6. *Naturvidenskabelig og Mathematisk Afdenling*, 10, 149–316.

Maron J.L., Combs J.K. & Louda S.M. (2002) Convergent demographic effects of insect attack on related thistles in coastal vs. continental dunes. *Ecology*, 83, 3382-3392.

Mcclay, A. S., R. Bourchier, R. A. Butts, AND D. P. Peschken (in Mason, P. G. AND J. T. Huber (EDS.). (2001) *Cirsium arvense* (L.) Scopoli, Canada thistle (Asteraceae), pp. 318-330. In Mason, P. G. and J. T. Huber (eds.). *Biological Control Programmes in Canada*, 1981-2000. 318-330.

McEvoy PB, R. N., Cox CS, Huso M (1993) Disturbance, Competition, and Herbivory Effects on Ragwort Senecio Jacobaea Populations. *Ecological Monographs*, 63, 55-75.

Mitchell, C. E. & Power, A. G. (2003) Release of invasive plants from fungal and viral pathogens. Nature, 421, 625-627.

Moore R.J. (1975) Biology of canadian weeds .13. *Cirsium-arvense*(l) scop. *Canadian Journal of Plant Science*, 55, 1033-1048.

Murdoch WW, C. J., Chesson PL (1985) Biological Control in Theory and Practice. *The American Naturalist*, 125, 344-366.

Myers J., Bazely D. (2003) Ecology and Control of Introduced Plants. Cambridge University
Press, Cambridge.

Nuzzo (1997) Element Stewardship Abstract For *Cirsium arvense*, The Nature Conservancy.

O‘Brien, C. W. and G. J. Wibmer. 1982. Annotated checklist of the weevils (Curculionidae sensu lato)of North America, Central America, and the West Indies (Coleoptera: Curculionoidea). American Entomological Institute, Ann Arbor, Michigan, USA. 382 pp.

Peschken, D. (1984) Host range of *Lema cyanella* (Coleoptera: Chrysomelidae), a candidate for biocontrol of Canada thistle, and of four stenophagous, foreign thistle insects in North America. *Canadian Entomologist*, 116.

Pywell, R. F., M. J. Hayes, J. B. Tallowin, K. J. Walker, W. R. Meek, C. Carvell, L. A. Warman, and J. M. Bullock. Minimizing environmental impacts of grassland weed management: can *Cirsium arvense* be controlled without herbicides? *Grass and Forage Science* 65:159-174.

Reed C.C., Larson D.L. & Larson J.L. (2006) Canada thistle biological control agents on two South Dakota wildlife refuges. *Natural Areas Journal*, 26, 47-52.

Rees, N.E., P.C. Quimby, G.L. Piper, E.M. Coombs, C.E. Turner, N.R. Spencer and L.V. Knutson. (1996) Biological Control of weeds in the West. Western Society of Weed Science, Bozeman Montana.

Schadler M., Jung G., Brandl R. & Auge H. (2004) Secondary succession is influenced by belowground insect herbivory on a productive site. *Oecologia*, 138, 242-252.

Eric C. Vasquez & Gretchen A. Meyer (2011) Relationships among leaf damage, natural enemy release, and abundance in exotic and native prairie plants, *Biological Invasions,* 13, 621-633.

Torchin M.E., Lafferty K.D. & Kuris A.M. (2002) Parasites and marine invasions. Parasitology, 124, S137-S151

Turnbull A.L. & Chant D.A. (1961) Practice and theory of biological control of insects in canada. *Canadian Journal of Zoology*, 39, 697-753.

Tiley, G. E. D. Biological Flora of the British Isles: *Cirsium arvense* (L.) Scop. Journal of Ecology, 98, 938-983.

web 1 (17.5.2011):http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb5201685.pdf.

Zwölfer, H. 1965. A list of phytophagous insects attacking wild Cynareae species in Europe. Progress Report 15. Commonwealth Institute of Biological Control, Silwood Park, Ascot, United Kingdom.

Figures and Tables

Figure 1. Effect of *C. rubiginosa* addition, distribution range and year of measurement on number of flower heads per plant.

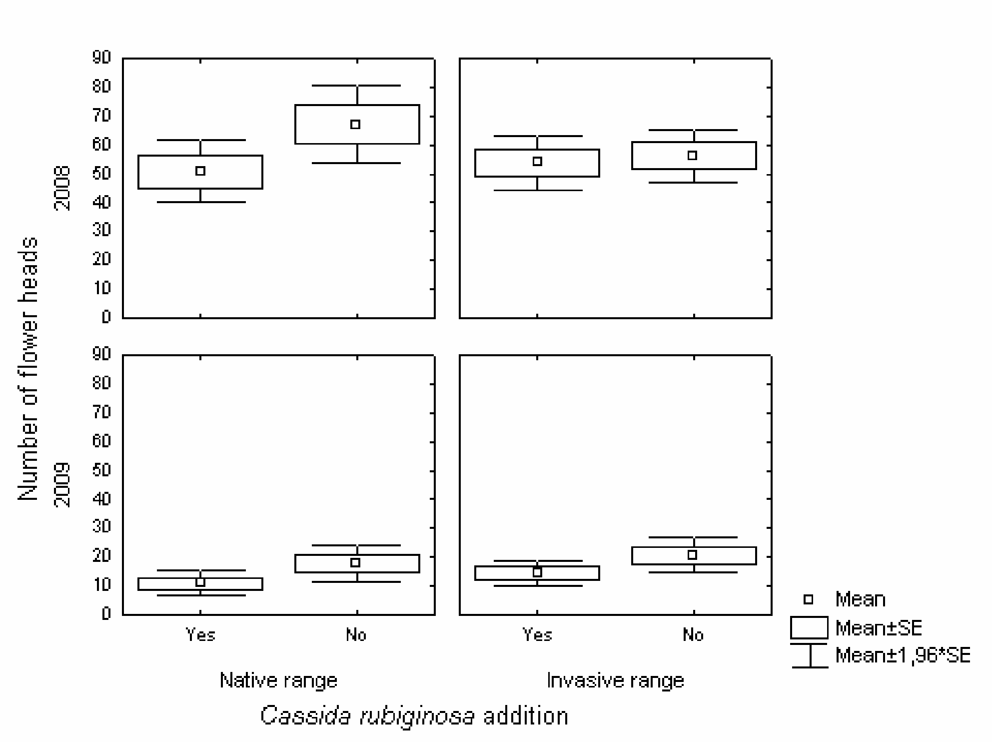

Figure 2. Effect of *R. conicus* addition, distribution range and year of measurement on flower head biomass.

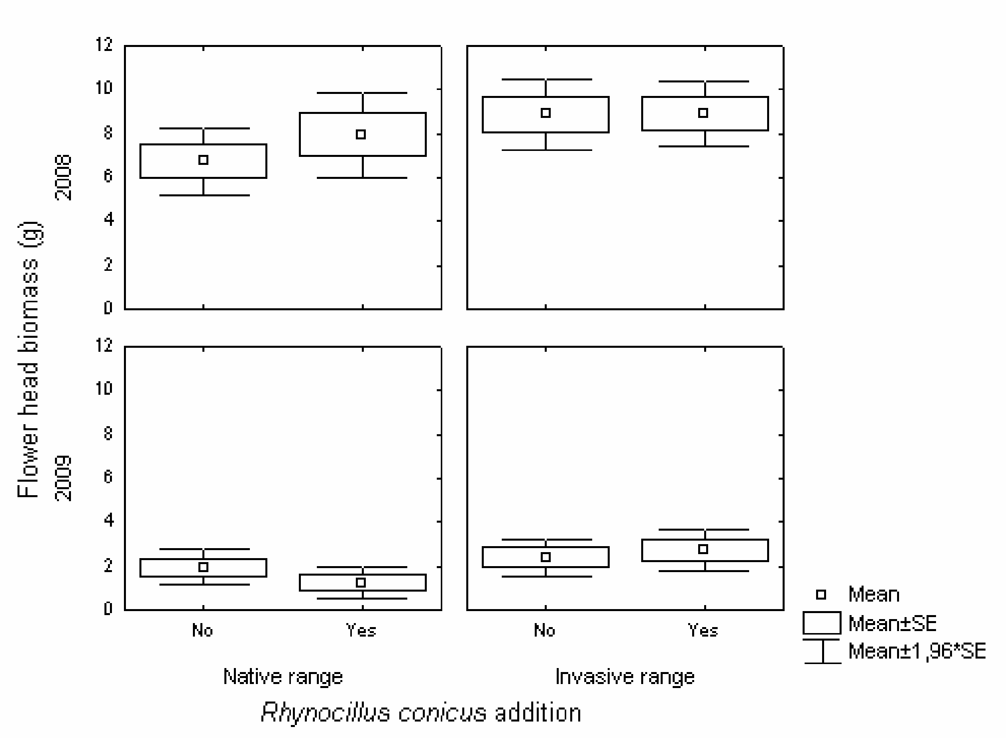

Figure 3. Effect of *U. cardui* addition, distribution range and year of measurement on flower head biomass.

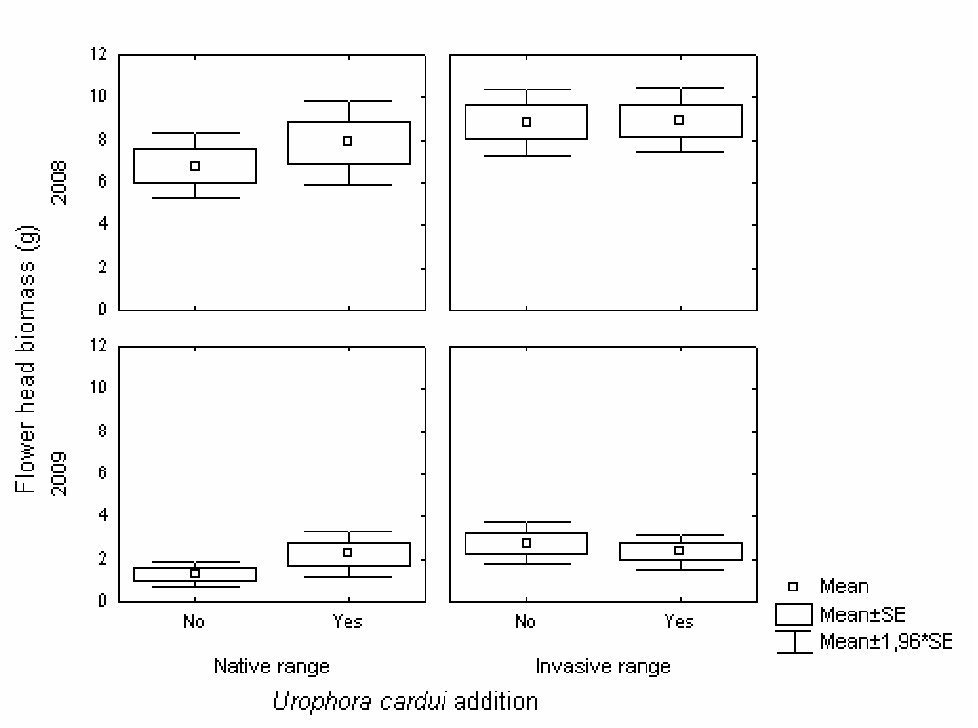

Figure 4. Effect of all insect addition treatments, distribution range and year of measurement on number of flower heads.

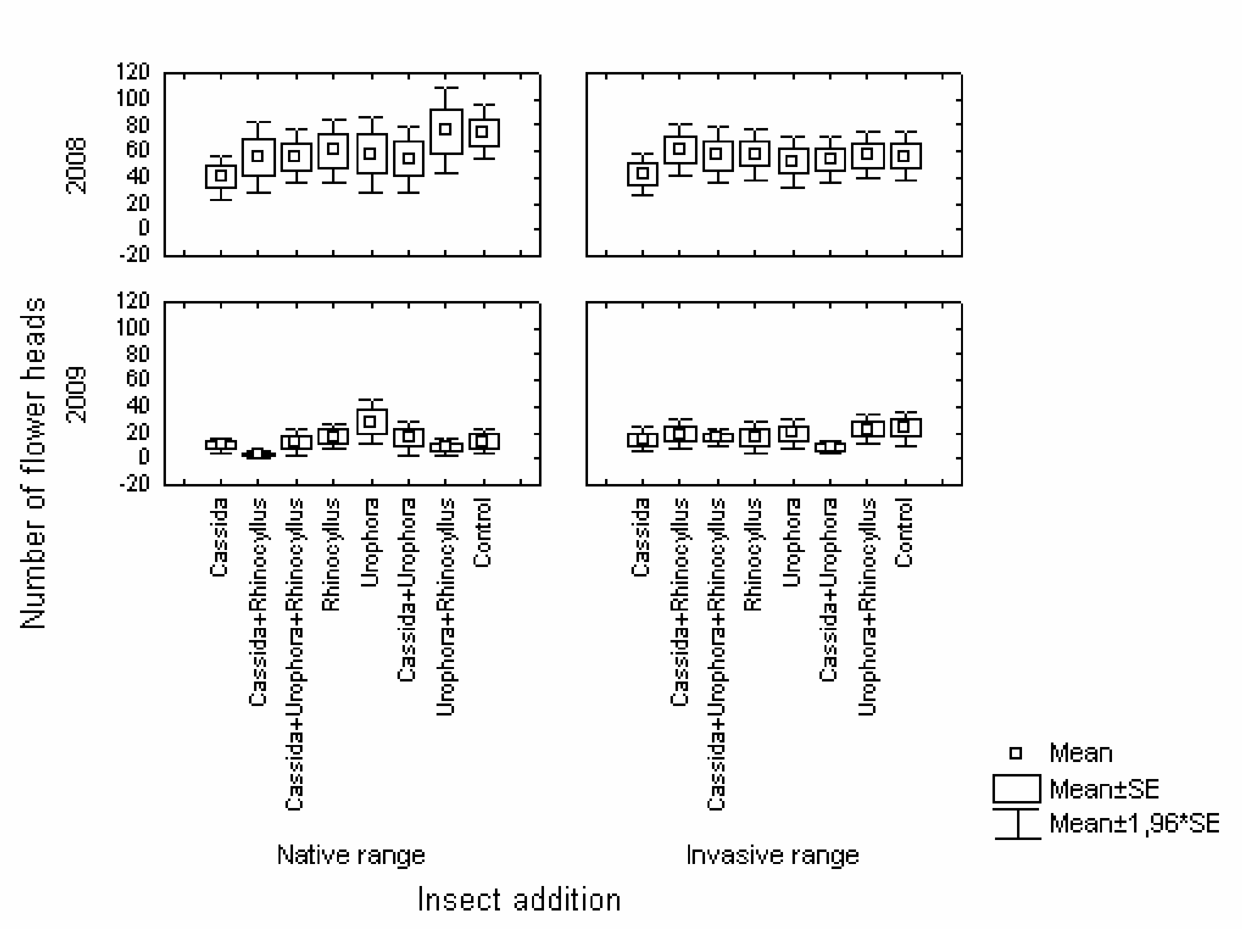

Figure 5. Effect of all aboveground insect addition, underground insect addition and aboveground+underground treatments, distribution range and year of measurement on aboveground biomass.

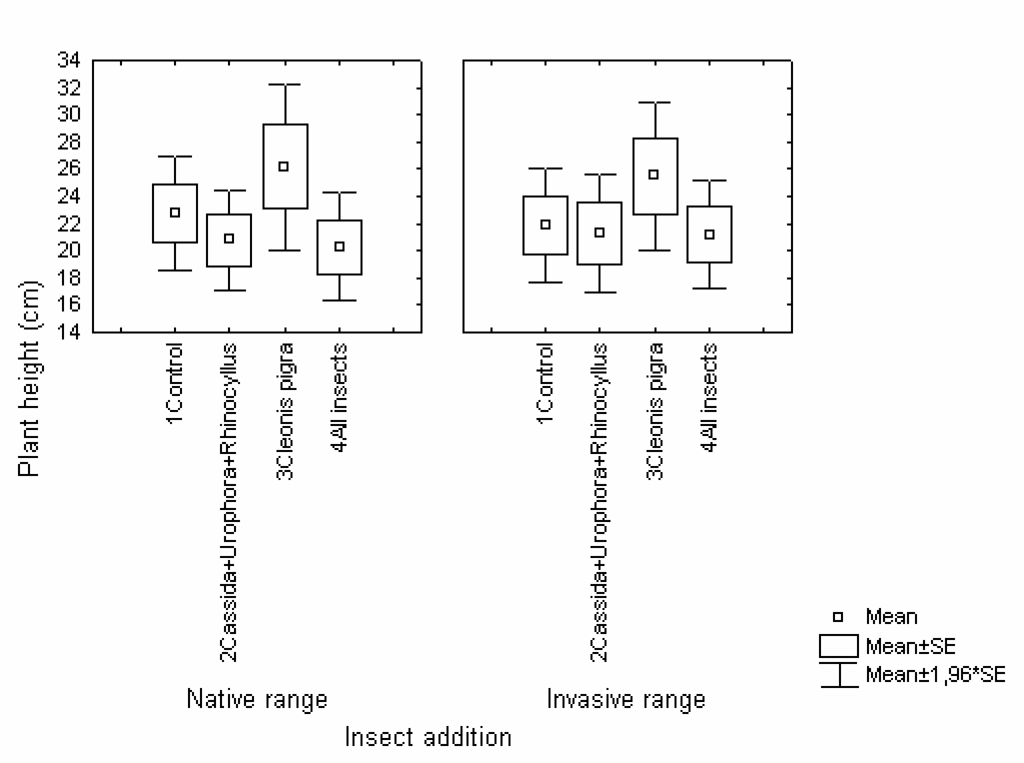

Table 1. Location and climate information from sample sites, where seeds for the herbivory experiment were collected.

Distribution range	Population	Altitude	Latitude (m.)	Climate
Native	Spain	N 41º 29' 40" E 1º 19' 27"	703	Short moderate winters, long, hot and dry summers, mediterranean continental climate
	Czech Republic	N 49º 51' 06" E 14º 12' 37"	460	Long, cold winters, short summers, continental climate.
Invasive	Nebraska	N 40 º 54' 00" W 96 º 23' 13" N	338	Cold winters, long, hot summers, humid continental climate.
	Illinois	N 41° 19' 16.10" W 89° 55' 58.99"	237	Cold winters, long, hot summers, humid continental climate

Table 2. Chemical composition of the two substrates used in the experiment. The values are mean values from 10 samples ($p < 0.001$ for all tests), standard errors are shown.

Substrate	g. kg-1 Nitrogen	g. kg-1 Total soil Carbon	g. kg-1 Soil carbon in Carbonates	g. kg-1 Soil organic Carbon	Phosphorus (mg/1000g)
Poor	0.72 Mean +- SE 0.003	1.21 Mean +- SE 16.473	0.04 Mean +- SE 0.003	1.14 Mean +- SE 16.474	62.14 Mean +- SE < 0.001
Rich	0.88 Mean +- SE 0.003	8.42 Mean +- SE 0.022	1.11 Mean +- SE 0.007	7.31 Mean +- SE 0.023	2.46 Mean +- SE 2.722

Table 3. Effect of individual species (*C. rubiginosa*, *R. conicus*, *U. cardui*), and combinations of these aboveground herbivore species in the insect addition experiment, with year of measurement, type of soil substrate and distribution range source of seed origin on *C. arvense* aboveground growth and reproduction. R^2 values for significant factors are shown, where *** means p-values < 0.0001, ** means p-values < 0.001 and * means p-values < 0.05.

	Height	Aboveground biomass	Flower head biomass	Flower head biomass	Number of flowers heads
Covariate				Aboveground biomass	
Cassida rubiginosa addition	n.s.	n.s.	n.s.	n.s.	n.s.
Rhynocillus conicus addition	n.s.	n.s.	n.s.	n.s.	n.s.
Urophora cardui addition	n.s.	n.s.	n.s.	n.s.	n.s.
Year * *C. rubiginosa* addition	n.s.	n.s.	0.008 **	0.008 **	0.012 ***
Range * Year * *R. conicus* addition	n.s.	n.s.	< 0.0001 *	0.003 *	n.s.
Range * Year * *U. cardui* addition	n.s.	n.s.	0.003 *	0.003 *	n.s.
Range * Substrate * *C. rubiginosa* * *R. conicus* * *U. cardui*	n.s.	n.s.	0.015 ***	0.015 **	0.007 **
Range * Substrate * Year * *C. rubiginosa* * *R. conicus* * *U. cardui*	0.002 *	n.s.	n.s.	n.s.	n.s.
Range * Year * *C. rubiginosa* * *R. conicus* * *U. cardui*	n.s.	0.001 *	0.013 ***	n.s.	0.004 *
Range * *C. rubiginosa* * *R. conicus* * *U. cardui*	n.s.	n.s.	n.s.	n.s.	0.007*
Year * *C. rubiginosa* * *R. conicus* * *U. cardui*	n.s.	n.s.	n.s.	n.s.	0.004 *
Range * *C. rubiginosa* * *R. conicus*	n.s.	n.s.	n.s.	n.s.	n.s.
Substrate * *C. rubiginosa* * *R. conicus*	n.s.	n.s.	0.003 *	0.003 *	0.004 *
Range * Substrate * *R. conicus* * *U. cardui*	n.s.	n.s.	0.003 *	n.s.	n.s.

Table 4. Effect of combined aboveground (*C. rubiginosa*, *R. conicus*, *U. cardui*) and underground (*C. pigra*) insect addition, year of measurement, type of substrate and seed range of distribution origin on *C. arvense* aboveground growth and reproduction. R^2 values for significant factors are shown, where *** means p-values < 0.0001, ** means p-values < 0.001 and * means p-values < 0.05

	Height	Aboveground biomass	Flower head biomass	Flower head biomass	Number of flower heads
Covariate				Aboveground biomass	
Aboveground insect addition	0,009 *	n.s.	n.s.	n.s.	n.s.
Underground insect addition	n.s.	n.s.	n.s.	n.s.	n.s.

Table 5.Effect of combined aboveground (*C. rubiginosa*, *R. conicus*, *U. cardui*) and underground (*C. pigra*) insect addition, year of measurement, type of substrate and seed range of distribution origin on *C. arvense* root growth and total plant biomass. R^2 values for significant factors are shown, where *** means p-values < 0.0001, ** means p-values < 0.001 and * means p-values < 0.05

	Df	Healthy root biomass	Damaged root biomass	Dead root biomass	Root length	Root:shoot ratio	Total plant biomass
Covariate			Healthy + damaged root biomass	Healthy + damaged + dead root biomass			
Aboveground insect addition	1	n.s.	n.s.	0.018 *	n.s.	n.s.	0.020 *
Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Distribution range : Aboveground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Substrate : Aboveground insect addition	1	n.s.	n.s.	0.015 *	n.s.	0.019 *	n.s.
Distribution range : Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Substrate : Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Aboveground insect addition : Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Country of origin : Aboveground insect addition	2	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Distribution range : Substrate : Aboveground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Country of origin : Underground insect addition	2	n.s.	0.015 *	n.s.	n.s.	n.s.	0.019 *
Substrate : Aboveground insect addition : Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Country of origin : Substrate : Aboveground insect addition	2	n.s.	0.035 ***	n.s.	n.s.	0.027 *	n.s.
Country of origin : Aboveground insect addition : Underground insect addition	2	n.s.	0.019 *	n.s.	n.s.	n.s.	n.s.
Distribution range : Substrate : Aboveground insect addition : Underground insect addition	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Country of origin:Distribution range : Substrate : Aboveground insect addition : Underground insect addition	2	n.s.	n.s.	n.s.	n.s.	0.025 *	n.s.

Appendices:

Appendix 1. Number of insects added per plant.

Year of addition	2008		2009	
Insects	Larvae	Adults	Larvae	Adults
Cassida rubiginosa	4		4	
Rhinocyllus conicus		4 (♂/♀)		4 (♂/♀)
Urophora cardui		4 (♂/♀)		4 (♂/♀)
Cleonis pigra				1 (♀)

Appendix 2. Effect of insect addition on plant height, aboveground biomass, flower head biomass, number of flower heads comparing with control plants in native and invasive range, poor and rich type of substrate, in 2008 and 2009 and in both ranges and substrates toghether (All groups). Only those parameters which resulted significant are shown in the table (but all growth and reproduction parameters were analysed in all insect treatments). Significant negative effect of the treatment on the parametr is shown by a "+" sign and significant positive effect of the treatment on the parametr is shown by a "-"sign.

A)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
Cassida rubiginosa	Flower head biomass	Invasive	+	0	0	0	+	0
Cassida rubiginosa	Flower head biomass	Native	0	0	0	0		
Cassida rubiginosa	Number of flower heads	Invasive	+	0	0	0	+	0
Cassida rubiginosa	Number of flower heads	Native	0	0	0	0		

B)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
Rhynocillus conicus	Plant height	Invasive	+	0	+	+	+	+
Rhynocillus conicus	Plant height	Native	0	0	+	+		
Rhynocillus conicus	Aboveground biomass	Invasive	0	+	0	0	0	0
Rhynocillus conicus	Aboveground biomass	Native	0	0	0	0		

C)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
Urophora cardui	Plant height	Invasive	+	+	+	0	+	0
Urophora cardui	Plant height	Native	+	-	0	0		
Urophora cardui	Aboveground biomass	Invasive	-	+	0	0	+	0
Urophora cardui	Aboveground biomass	Native	+	-	0	0		
Urophora cardui	Flower head biomass	Invasive	+	+	0	0	+	0
Urophora cardui	Flower head biomass	Native	+	-	0	0		
Urophora cardui	Number of flower heads	Invasive	+	+	0	0	+	0
Urophora cardui	Number of flower heads	Native	+	-	0	0		

D)

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
Cleonis pigra	Plant height	Invasive	0	+	+
Cleonis pigra	Plant height	Native	0	+	

E)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
C.rubiginosa + *R.conicus* + *U.cardui*	Plant height	Invasive	0	+	0	+	0	+
C.rubiginosa + *R.conicus* + *U.cardui*	Plant height	Native	0	0	0	0		
C.rubiginosa + *R.conicus* + *U.cardui*	Aboveground biomass	Invasive	0	+	0	0	+	0
C.rubiginosa + *R.conicus* + *U.cardui*	Aboveground biomass	Native	0	0	0	-		

F)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
Aboveground insects	Plant height	Invasive	0	0	0	0	0	0
Aboveground insects	Plant height	Native	0	-	0	0		
Aboveground insects	Aboveground biomass	Invasive	0	0	0	0	0	+
Aboveground insects	Aboveground biomass	Native	0	0	0	0		

G)

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
Aboveground insects + *C.pigra*	Plant height	Invasive	0	0	+
Aboveground insects + *C.pigra*	Plant height	Native	0	+	
Aboveground insects + *C.pigra*	Aboveground biomass	Invasive	+	0	0
Aboveground insects + *C.pigra*	Aboveground biomass	Native	0	0	

H)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
C.rubiginosa + *R.conicus*	Plant height	Invasive	+	-	+	+	0	+
C.rubiginosa + *R.conicus*	Plant height	Native	0	0	-	+		
C.rubiginosa + *R.conicus*	Aboveground biomass	Invasive	+	0	0	0	0	0
C.rubiginosa + *R.conicus*	Aboveground biomass	Native	0	0	0	+		

I)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
C.rubiginosa + *U.cardui*	Plant height	Invasive	+	0	0	0	0	0
C.rubiginosa + *U.cardui*	Plant height	Native	0	0	0	+		
C.rubiginosa + *U.cardui*	Aboveground biomass	Invasive	+	0	0	0	0	0
C.rubiginosa + *U.cardui*	Aboveground biomass	Native	0	0	0	+		

J)

Treatment	Parameter	Distribution range	Poor 2008	Rich 2008	Poor 2009	Rich 2009	All groups 2008	All groups 2009
R.conicus + *U.cardui*	Aboveground biomass	Invasive	+	0	0	0	0	0
R.conicus + *U.cardui*	Aboveground biomass	Native	0	0	0	+		

K) Effect of aboveground insect, *Cassida r.*, *R.conicus*,*C. rubiginosa*+*R. conicus, C. rubiginosa*+*U. cardui R. conicus*+*U. cardui* addition on healthy root biomass, damaged root biomass, root lenght, root:shoot ratio and total plant biomass comparing with control plants in native and invasive range, poor and rich type of substrate, in 2009 and in both ranges and substrates toghether (All groups). Only those parameters which resulted significant are shown in the table (but all growth and reproduction parameters were analysed in all insect treatments). Significant negative effect of the treatment on the parametr is shown by a "+" sign and significant positive effect of the treatment on the parametr is shown by a "-"sign.

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
C.rubiginosa + R.conicus + U.cardui	Healthy root biomass	Invasive	+	0	0
C.rubiginosa + R.conicus + U.cardui	Healthy root biomass	Native	0	0	
C.rubiginosa + R.conicus + U.cardui	Damaged root biomass	Invasive	+	0	+
C.rubiginosa + R.conicus + U.cardui	Damaged root biomass	Native	0	0	
C.rubiginosa + R.conicus + U.cardui	Root lenght	Invasive	+	0	+
C.rubiginosa + R.conicus + U.cardui	Root lenght	Native	0	0	
C.rubiginosa + R.conicus + U.cardui	Root:shoot ratio	Invasive	0	0	+
C.rubiginosa + R.conicus + U.cardui	Root:shoot ratio	Native	+	0	
C.rubiginosa + R.conicus + U.cardui	Total plant biomass	Invasive	0	0	+
C.rubiginosa + R.conicus + U.cardui	Total plant biomass	Native	0	0	

L)

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
Cassida rubiginosa	Healthy root biomass	Invasive	0	0	0
	Healthy root biomass	Native	0	+	

M)

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
Rhynocillus conicus	Root:shoot ratio	Invasive	0	0	0
	Root:shoot ratio	Native	+	0	

N)

Treatment	Parameter	Distribution range	Poor 2009	Rich 2009	All groups 2009
C.rubiginosa + *U.cardui*	Healthy root biomass	Invasive	0	0	+
C.rubiginosa + *U.cardui*	Healthy root biomass	Native	0	+	
C.rubiginosa + *U.cardui*	Root lenght	Invasive	0	0	+
C.rubiginosa + *U.cardui*	Root lenght	Native	+	0	
C.rubiginosa + *U.cardui*	Root:shoot ratio	Invasive	0	0	0
C.rubiginosa + *U.cardui*	Root:shoot ratio	Native	0	+	
C.rubiginosa + *U.cardui*	Total plant biomass	Invasive	0	0	0
C.rubiginosa + *U.cardui*	Total plant biomass	Native	0	0	

CHAPTER 4

Increased performance of *Cirsium arvense* from the invasive range

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Chapter 4

Increased performance of *Cirsium arvense* from the invasive range

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Abstract

The Evolution of Increased Competitive Ability hypothesis (EICA) suggests that plants from the invasive range should perform better than plants of the same species from the native range. To properly test this, we need to compare growth of plants from the two ranges in a common environment. Ideally, all the natural enemies should be excluded, to make sure that the differences are not due to different response of plants from the two ranges to the natural enemies. We used the above design to examine the difference in growth and reproduction in *Cirsium arvense* plants from the invasive (North America) and native range (Europe). To account for possible differences within the ranges, we used plants from two regions, separated by at least 1000 km, in each range. Because the higher performance of species from the invasive range can be caused by their higher ability to acquire resources we compared growth of the plants in two different nutrient levels. The results indicate that plants from the invasive range are larger in most size parameters as well as parameters more closely related to fitness. For aboveground biomass, the response of plants from the invasive range to nutrient addition was weaker than that of plants from the native range and the difference between the ranges was stronger in the nutrient poor substrate. The results are in agreement with the EICA hypothesis and suggest that plants from the invasive range have higher ability to use resources and are thus able to perform well also in nutrient poor conditions.

Keywords: Canada thistle, noxious weeds, Europe, North America, clonal growth, EICA hypothesis.

Introduction

Plant invasion is an important problem for natural and agronomic systems, and a major threat to global biodiversity (Vitousek, 1990; Wilcove et al. 1998). There is a wide range of hypotheses on the possible mechanisms of plant invasiveness (Catford et al. 2009). To understand these mechanisms, comparative studies including invasive species are needed (Williams et al. 2009; van Kleunen et al. 2010). The most commonly performed types of studies are those comparing plants invasive and native to a given area, or non-native, invasive species with non-native, non-invasive species that are naturalized (e.g. Jeschke and Strayer 2006; Pysek and Richardson 2007; Garcia-Serrano et al., 2009), or populations of the same species from the native and invasive range (e.g. Bossdorf et al 2005; Zou et al. 2007; Colautti et al. 2009).

Comparing populations of the same species from the native and invasive range is the major type of the study that can be used to test the Evolution of Increased Competitive Ability hypothesis (EICA), which predicts that plants in its invasive range allocate more to growth than to defense (Blossey and Nötzold, 1995). As a result, populations in the invasive range have improved competitive abilities. The Enemy Releases Hypothesis (ERH) originated as an extension of the EICA (Keane and Crawley, 2002). This hypothesis says that the invasive potential of some non-indigenous species may be enhanced due to absence of natural enemies such as specialist herbivores in the introduced range (e.g., Keane and Crawley 2002; Torchin and Kuris 2002; Mitchell and Power 2003; Colautti et al. 2004; Carpenter and Cappuccino 2005). According to this hypothesis, growth of invasive plants in the introduced range will be higher than that in the native range due to absence of natural enemies. To identify if the differences between the ranges are due to ERH, or if there are additional differences suggested by the EICA, it is crucial to grow plants from the two ranges in a common environment. As demonstrated by Williams et al. (2008), conclusions on the possible differences between the native and invasive range depend on the environment in which the plants are cultivated. It is thus important to perform such an experiment in more than one type of environments.

Despite the fact that herbivores are predicted to be the main agents responsible for the differences in performance between the two ranges, most of the experiments were carried out in common gardens and plants were thus exposed to native herbivores. However, in a strict experimental approach, natural herbivores such as insects and mammal herbivores should be excluded (van Kleunen and Schmid 2003; Blair and Wolfe 2004; Williams et al. 2008).

In this study, we compared growth of *Cirsium arvense* from the invasive and native range to see if the plants from the invasive range showed signs of increased competitive ability. *C. arvense* has many natural enemies, such as the insect *Cassida rubiginosa* (Kruess, 2002), which attacks leaves, aphids and nematodes (Bezemer et al., 2004), sap-sucking herbivores, or fungal pathogens (Guske et al., 2004) Aboveground natural enemies were excluded in our experiment. However, we did not exclude all the soil natural enemies as not all the soil used for the experiment could be sterilized. We thus studied performance of the plants in absence of aboveground enemies and with reduced amount of belowground enemies.

Common garden studies are increasingly used to identify differences in phenotypic traits between native and introduced genotypes, but they often ignored possible differences among populations within each range (Colautti et al., 2009). To take this into account, we used plants from two distant countries/states from each range.

Higher performance in the experiment of plants from the invasive range can be caused by their higher ability to acquire resources (Erfmeier and Bruelheide, 2004) and the outcome of the experiment may depend on the conditions under which the plants are cultivated (Williams et al., 2008). We thus compared growth of the plants under two different nutrient levels under controlled conditions.

Specifically, we addressed the following questions: (i) Do plants of *Cirsium arvense* from the native and invasive range differ in growth and reproduction? (ii) Are there any interactions between plant origin (native and invasive range) and the type of substrate? We predict that plants from the invasive range grow better and show overall higher fitness, as predicted by EICA. We also predict that plants from the invasive range have better ability to use resources even in nutrient poor conditions. Therefore, their responses to nutrient addition should be weaker than that of plants from the native range.

Material and methods

Study system

Cirsium arvense (L.) Scop. (Asteraceae) is a perennial herb with prickly leaves, erect stems 0.5 – 1.0 m tall, and an extensive creeping rootstock (Nuzzo, 1997). It is a dioecious species and also reproduces by clonal growth. *C. arvense* is native to south-eastern Europe and the eastern Mediterranean (Moore, 1975), possibly also to northern Europe, western Asia and northern Africa (Detmers, 1927; Amor and Harris, 1974). At least, it is an archeophyte there. This species was introduced to North America in the 1600's as a contaminant of grain seed (Jacobs et al., 2006). It is also invasive in parts of Africa, the Middle East, India, Japan, New Zealand, Australia and South America (Julien and Griffiths, 1998). It is one of the most problematic weeds in North America (Moore, 1975; Pritekel et al., 2010), where there was an attempt to manage its invasion by classical biological control (Schroeder, 1980; Peschken, 1984; McClay et al., 2001). The species is considered dioecious, but in fact many individuals are hermaphrodite (Kay 1985).

Sampling sites

Cirsium arvense seeds were collected in June, July and August 2007 in two continents, representing the native and invasive range of the species (more information on sites in Table 1). All the four sampling sites were abandoned fields in agricultural landscapes. Using this design we covered large parts of both the native and invasive range, with populations in the native range being 2000 km apart and populations in the invasive range being 1200 km apart. The native and invasive ranges are 7900 km apart.

<<< Tab. 1

Garden experiment

From each distribution range (invasive/native) we used plants from two countries as described above and five mother plants per country each selected from different populations if possible. Offspring (seeds) of these mother plants were grown in 2 substrates resulting in 2 distribution ranges x 2 countries x 2 substrates x 5 replicates in total.

During autumn of 2007, the collected flowerheads from all natural populations were dissected and seeds stored in paper bags. At the end of January 2008, seeds were soaked in 10 g L^{-1} $KMnO_4$ solution for 10 min to prevent fungal infestation. Seeds were placed in pots filled with sand and covered with a metal net with 1 mm mesh size to avoid insect feeding. These pots were buried underground in an experimental garden for stratification to enhance seed germination in spring. In March 2008, seeds were extracted from the sand and five seeds each were sowed in 5 cm x 5 cm pots in a greenhouse without heating. After three weeks, we kept one well established seedling in each pot and removed the others. The seedlings were planted in a common garden in April 2008.

The experimental garden was situated in the Czech Republic (N49° 59' 40" E14° 33', 368 m altitude), relatively close to the area from which the Czech populations were sampled. Plants from the Czech Republic were thus grown in the conditions which were very similar to conditions of the populations from which the seeds were collected, while plants from the other countries were grown in conditions which differed from conditions from which the seeds were collected. It would be ideal to follow growth of all plants in at least one more experimental garden, ideally situated in the invasive range. Although this was originally planned, it unfortunately could not be realized. The root system of *C. arvense* is very large. Roots and rhizomes were mixed, these will be defined as “roots” in the text. Because we wanted to make sure that each plant had enough space to grow and also to fully control the environment in which the plants were grown, we could not plant the seedlings directly into the soil in the garden. Instead, we transplanted the seedlings into large pots, each made of two plastic bags (100 cm long x 75 cm in diameter, 2 mm thick). We used two bags for each pot to make sure that it did not tear easily. These pots were filled with 50 L substrate in April 2008. One 0.5 cm x 0.5 cm hole was made at the bottom in every bag to avoid excessive soil moisture. To study the effect of soil conditions on plant growth, two types of substrates were used. For the nutrient poor substrate, the bags were filled with sand from a quarry 100 km away from the experimental site. For the nutrient rich substrate, the bags were filled with soil from the nearby garden.

To describe the differences between the two substrates used in the experiment, ten samples from each substrate type were taken in May 2010, one from each bag. Content of nitrogen, total carbon, carbonate carbon, organic carbon and phosphorus were measured in the laboratory of the Institute of Botany, Academy of Sciences of the Czech Republic. Content of total N and total C were analyzed following Ehrenberger and Gorbach (1973), carbonate content was analyzed according to ISO-Standard (10693) and available P was determined using the photometric methods of Olsen et al. (1954) and Olsen and Sommers (1982). The differences in soil conditions are described in Table 2.

<<< Tab. 2

The lowermost third of each bag was filled with gravel (1 cm in diameter) to assure drainage. Due to the high volume of substrate, all of it could not be sterilized. To limit germination of seeds of various weedy species from the non-sterile soil, 6 cm layer of a mixture of 10:1 sterilized sand and perlite was placed on the top of the substrate. Adding such a layer strongly limits germination of weeds as most of the possible weedy species require full light for germination (pers. obs.).

Immediately after planting the seedlings, cylindrical metal constructions, 39 cm in diameter and 98 cm high, were placed on the soil on each plant. White translucid polyamide sacks were placed over each metal construction and fastened to the outer part of the bags. This was done to make sure that no natural enemies were able to attack the plants. Radiation under the mesh was measured with a SPh 2020 photometer from Optické dílny Turnov, CR. The mesh decreased PAR (photosynthetic active radiation) by 11.7%. It also changed somewhat temperature and humidity. However, this was given for all the studied plants in the same intensity, so that this modification of the environment did not affect differently the resulting growth differences of the plants.

Plant growth was measured in the time of full flowering in August in 2008 and 2009, respectively. Data of all plants were sampled within two days. Plant measurements included plant height, number of stems and number of flowerheads. Each year after the measurements all aboveground biomass was harvested, dried to a constant weight, and weighed. Because the species is dioecious in principle and, indeed, about half of the plants were male, it was not possible to evaluate seed production of all the plants. Number of flowerheads per plant can be considered as a fitness measure since it strongly positively correlates with number of seeds/plant in the female plants (R^2= 0.62).

During May 2010, the experimental plants were harvested. Roots were carefully extracted from the soil and cleaned with water to remove all the substrate. Three categories of roots were differentiated: healthy roots, damaged roots, and dead roots. Healthy roots were white. Roots were classified as damaged when they had brown color or had other clear signs of damage. Roots were considered as dead when they were dark brown and decomposing. Whereas above ground plant parts were isolated from herbivores with a mesh, soil pathogens could have been cause of the damage because the plants were planted in a non-sterile soil. The length of the healthy parts of roots wider than 1 mm was measured with a rule. Healthy, damaged and dead roots were weighed before and after drying to a constant weight.

Data analysis

To analyze the effect of plant origin (invasive or native range and country nested within range), soil type and year of measurement on plant growth, we used generalized linear models in S-Plus (2000). Plant height, aboveground biomass, number of stems and flowerhead biomass were taken as dependent variables and distribution range (invasive/native), country (nested within distribution range), soil type and year as independent variables. In case of flowerhead biomass, total aboveground biomass was used as a covariate. In this way, we studied differences in biomass allocation to flowerheads rather than differences in biomass of the flowerheads. The same analysis without covariate was also done. Aboveground biomass, flowerhead biomass and plant height was analyzed using GLM with Gamma distribution, as their distribution is also strongly deviating from normal distribution and is right skewed (Crawley 2002).

Data on root biomass, root length and root to shoot ratio were available only at the end of the experiment (in 2010). The independent variables in the root data analyses were distribution range (invasive/native), country (nested within distribution range) and soil type. Healthy root biomass, damaged root biomass, dead root biomass, length of healthy roots root to shoot ratio (total root biomass/aboveground biomass) and total plant biomass (aboveground biomass + root biomass) was analyzed using GLM assuming Gamma distribution. We also analyzed the proportion of damaged roots by using biomass of damaged roots as dependent variable and biomass of healthy root plus damaged roots as a covariate.

Results

Distribution range had a significant effect on aboveground biomass (Table 3) with plants from the invasive range being larger (Fig. 1). Country of origin nested within distribution range had a significant effect on aboveground biomass and number of stems (Table 3). Aboveground biomass and number of stems of the plants from the Czech Republic and Nebraska were relatively larger (after accounting for the main effect of distribution range) than those from Spain and Illinois (Table 3). Compared with the plants from the nutrient poor soil, the plants grown in the nutrient rich soil were significantly larger in all size parameters except for healthy root biomass (Table 3 and 4, Fig. 2). Flowerhead biomass (Fig. 3), number of stems and plant height (Fig. 4) were higher in 2009 than in 2008 (Table 3).

<<< Tab. 3

<<< Fig. 1 *(~1/4 height of page)*

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<<< Fig. 4 *(~1/4 height of page)*

Aboveground biomass and number of stems were also significantly affected by the interaction between distribution range and soil type (Table 3). For aboveground biomass, plants from the native range were significantly smaller than plants from the invasive range in nutrient poor soil, but not in the nutrient rich soil (Figure 1). Number of stems was significantly different between nutrient poor and rich substrate for plants from the native range, but not for plants from the invasive range (Figure 5). In both cases this indicates that plants from the native range responded more strongly to nutrient addition than plants from the invasive range.

Biomass allocation to flowerheads was affected by soil type and year (Table 3). Plants invested more biomass to flowerheads in 2008 than in 2009 and in poor substrate than in rich substrate (Fig. 3).

<<< Tab. 4

Distribution range and country significantly affected healthy root biomass (Table 4), and plants from the invasive range had higher healthy root biomass than plants from the native range (Fig. 2). Proportion of damaged roots was significantly affected by country of origin, soil type and by the country and substrate interaction, but not by distribution range (Table 4). Root length was significantly affected only by soil type (Table 4), and plants growing in nutrient poor substrate had longer roots than those in nutrient rich substrate. Soil type also affected root to shoot ratio (Table 4), and it was higher in nutrient poor and nutrient rich substrate. Total plant biomass (aboveground + root biomass) was affected by soil type as well (Table 4). Plants growing in rich substrate had higher total biomass than those growing in the poor soil type (Fig. 6).

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<<< Fig. 6 *(~1/4 height of page)*

Discussion

Cirsium arvense plants from North America (invasive range) grew larger than plants from the native range. Given that our studied plants were protected from the majority of herbivores, this result is consistent with the EICA hypothesis (Abilasha and Joshi, 2009). Even though the plants were protected from aboveground natural enemies, the plants were not fully protected from belowground enemies as they were grown in non- sterile soil. Indeed, we could identify this damage as damaged roots. No difference in proportion of damaged roots was, however, identified between the two ranges. This suggests that the plants from the two ranges did not differ in their ability to defend themselves against soil pathogens. As the main natural enemies of *C. arvense* are active aboveground (Fitter and Peat, 1994) as well as the main defense mechanisms are directed towards the aboveground natural enemies (Jordon-Thaden and Louda, 2003), this result does not exclude the validity of the EICA hypothesis. We thus conclude that the differences in growth of plants between the native and the invasive range are genetically based (Blossey and Nötzold, 1995).

Our results are comparable to the results of Erfmeier and Bruelheide (2004) who studied native and invasive populations of *Rhododendron ponticum* and demonstrated that plants from the invasive populations are higher and have more leaves and bigger stem diameter than plants from the native populations. Abhilasha and Joshi (2009) demonstrated that *Conyza canadensis* produced more reproductive biomass in the invasive range. Also Williams et al. (2008) found that growth was higher in the plants from the introduced range in the monocarpic perennial *Cynoglossum officinale*. However, in this case this was true only when the plants were grown in the garden in the native range and not in the invasive range. It could thus be argued that if we had performed our experiment also in the invasive range, we might have concluded that there are no differences in size between plants from the two ranges and would reject the EICA hypothesis as an explanation for invasiveness of *C. arvense*. Williams et al. (2008) did not provide a good explanation for the contrasting pattern in their study. We suggest that the major difference in their study could be due to presence of different natural enemies in the two ranges, as natural enemies were not excluded from their experiment. In our experiment, we excluded the majority of natural enemies (except for those present in the soil) and cultivated plants in two very different soil conditions. We might thus have had a better chance to uncover the genetic differences in size of plants from the two ranges. However, also in our study the possibility of a large genotype x environment interaction cannot be fully excluded.

In addition to the large differences in size of the plants from the native and invasive range, there were also significant differences between plant growth from the different countries. This is due to the large distances between the sampled countries within both ranges. Colautti et al. (2008) re-analyzed a range of studies comparing native and invasive populations of different species and demonstrated strong variation in performance of plants from both native and invasive range along a latitudinal gradient. In our study, the latitudinal gradient was too short (only about 9°) and we had worked only with four regions in total and we are thus not able to explain the differences in performance between our studied countries. Finding significant differences between ranges in spite of the large differences between countries within ranges suggests however, that the differences between ranges are strong.

Differences in growth of *C. arvense* from the native and invasive range were also found by Cripps et al. (2010) in a field survey in Europe, North and South New Zealand (native/invasive). Specifically, they found higher performance of plants in the South Island of New Zealand when compared to Europe. There were, however, no significant differences when comparing plants from North Island of New Zealand to Europe. Because they compared plants only in the field, without any transplantation, it is hard to say if the observed differences are due to real differences between the plants or due to different habitat conditions.

In our study, we found higher values for plants from the invasive range not only for the aboveground biomass but also for the root biomass. This contrasts to the conclusions of Zou et al. (2007) – and references therein – who found that Chinese tallow tree plants from the native range had more root biomass than plants from the invasive range. Ehrenfeld (2003) proposed that lower root to shoot ratio is closely associated with the increased size of invasive plants. On the other hand, Barney et al. (2009) demonstrated higher root to shoot ratio in *Artemisia vulgaris* from invasive populations than from native ones. In our study, we did not find any significant differences in root to shoot ratio between the two regions, and the amount of roots thus proportionally increased with increasing aboveground biomass. Also root length was independent of distribution range. In agreement with theoretical expectations, roots grew longer in poor nutrient substrate.

Our study also supported the expectation that plants from the invasive range have a higher ability to acquire resources. As a result the difference between plants from the native and invasive range was larger in the nutrient poor soil. This conclusion contrasts with the results of Williams et al. (2008) who found larger differences between native and invasive plants in the more productive conditions (in the garden in the native range) than in the low nutrient conditions (in the garden in the invasive range). It is, however,0 in agreement with studies of Drenovsky et al. (2008) and Allred et al. (2010), who confirmed better ability to acquire resources in invasive populations. The higher ability to acquire resources may be linked to larger belowground biomass of plants from the invasive range.

Rajaniemi and Reynolds (2004) and Drenovsky et al. (2008) demonstrated that plants with larger root systems also had higher precision in locating nutrients. Thanks to this the invasive plants may be able to capture more resources disproportionate to their size in spite of a constant root to shoot ratio. This can potentially promote size-asymmetric competition belowground and give the invasive plants strong advantage in the competition with native plants.

To sum up, our results demonstrated higher performance of *C. arvense* from the invasive range in aboveground biomass. In this clonal species, ability of clonal growth is an important measure of fitness. We found significantly higher amount of roots in the plants from the invasive range. Because roots and rhizomes are the organs of clonal growth, this indicates that also this measure of fitness is higher in the plants from the invasive range.

An optimal design of such a comparative experiment as the one presented here would be to growth both plants from the native range and from the invasive range in common garden conditions in both ranges (van Kleunen et al., 2010). This was unfortunately not feasible in our experiment and we grew plants only in the home range. Due to possible local adaptation it could be expected that plants would perform better when grown in their native range (e.g. Linhart and Grant, 1996, Kawecki and Ebert, 2004 Raabova et al., 2007). Higher fitness of plants from the invasive range found in our study thus suggest that the differences must be really large, as we can expect that plants from the invasive range would be even larger when all plants were grown in the invasive range.

Chapter 4

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References

Abhilasha, D., Joshi, J., 2009. Enhanced fitness due to higher fecundity, increased defense against a specialist and tolerance towards a generalist herbivore in an invasive annual plant. J. Plant Ecol. 2, 77–86.

Allred, B.W., Fuhlendorf, S.D., Monaco, T.A., Will, R.E., 2010. Morphological and physiological traits in the success of the invasive plant *Lespedeza cuneata*. Biol. Invas. 12, 739–749.

Amor, R.L., R.V. Harris, 1974. Distribution and seed production of *Cirsium arvense* (L.) Scop. in Victoria, Australia. Weed Res. 14, 317–323.

Barney, J.N., Whitlow, T.H., Ditommaso, A., 2009. Evolution of an invasive phenotype: shift to belowground dominance and enhanced competitive ability in the introduced range. Plant Ecol. 202, 275–284.

Bezemer, T.M., Graa, O., Rousseau, P., van der Putten, W.H., 2004. Above- and belowground trophic interactions on Creeping Thistle (*Cirsium arvense*) in high- and low-diversity plant communities: Potential for biotic resistance? Plant Biol. 6, 231–238.

Blair, A.C., Wolfe, L.M., 2004. The evolution of an invasive plant: An experimental study with *Silene latifolia*. Ecology 85, 3035–3042.

Blossey, B., Nötzold, R., 1995. Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. J. Ecol. 83, 887–889.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E., Prati, D., 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144, 1–11.

Cappuccino N., Carpenter, D., 2005. Invasive exotic plants suffer less herbivory than non-invasive exotic plants. Biol. Letters 1, 435–438.

Catford, J.A., Jansson, R., Nilsson, C., 2009. Reducing redundancy in invasion ecology by integrating hypotheses into a single theoretical framework. Divers. Distrib. 15, 22–40.

Cripps, M.G., Edwards, G.R., Bourdot, G.W., Saville, D.J., Hinz, H.L., Fowler, S.V., 2009. Effects of pasture competition and specialist herbivory on the performance of *Cirsium arvense*. Biocontrol Sci. Technol. 20, 641–656.

Colautti, R.I., Ricciardi, A., Grigorovich, I.A., Macisaac, H.J., 2004. Is invasion success explained by the enemy release hypothesis? Ecol. Lett. 7, 721–733.

Colautti, R.I., Maron, J.L., Barrett, S.C.H., 2009. Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. Evol. Applicat. 2, 187–199.

Crawley, M.J., 2002: Statistical Computing: An Introduction to Data Analysis using S-Plus. Wiley, Chichester.

Drenovsky, R.E., Martin, C.E., Falasco, M.R., James, J.J., 2008. Variation in resource acquisition and utilization traits between native and invasive perennial forbs. Am. J. Bot. 95, 681–687.

Ehrenberger, F., Gorbach, S., 1973. Methoden der organischen Elementar- und Spurenanalyse. Verlag Chemie, Weinheim.

Ehrenfeld, J. G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6, 503–523.

Erfmeier, A., Bruelheide, H., 2004. Comparison of native and invasive *Rhododendron ponticum* populations: Growth, reproduction and morphology under field conditions. Flora 199, 120–133.

Fitter, A.H., Peat, H.J., 1994. The Ecological Flora Database. J. Ecol., 82, 415–450.

Garcia-Serrano, H., Caño, L., Escarré, J., Fleck, I., Sans, F.X., 2009. Physiological comparison of alien *Senecio inaequidens* and *S. pterophorus* and native *S. malacitanus*: Implications for invasion. Flora 204, 445–455.

Guske, S., Schulz, B., Boyle, C., 2004. Biocontrol options for *Cirsium arvense* with indigenous fungal pathogens. Weed Res. 44, 107–116.

Hawkes, C.V., 2007. Are invaders moving targets? The generality and persistence of advantages in size, reproduction, and enemy release in invasive plant species with time since introduction. Am. Natural. 170, 832–843.

Jeschke, J.M., Strayer, D.L., 2006. Determinants of vertebrate invasion success in Europe and North America. Global Change Biol. 12, 1608–1619.

Julien, M.H., Griffiths, M.W., 1998. Biological Control of Weeds. A World Catalogue of Agents and their Target Weeds. 4th edit. CABI, Wallingford, UK.

Jordon-Thaden, I.E., Louda, S.A., 2003. Chemistry of *Cirsium* and *Carduus*: a role in ecological risk assessment for biological control of weeds? Biochem. Syst. Ecol., 31, 1353–1396.

Kruess A., 2002. Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*. Oecologia, 130, 563–569.

Kawecki, T.J., Ebert, D., 2004. Conceptual issues in local adaptation. Ecol. Lett. 7**,** 1225–1241.

Kay, Q.O.N., 1985. Hermaphrodites and subhermaphrodites in a reputedly dioecious plant, *Cirsium arvense* (L.) Scop. New Phytol. 100, 457–472.

Keane, R.M., Crawley, M.J., 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17, 164–170.

Linhart, Y.B., Grant, M.C., 1996. Evolutionary significance of local genetic differentiation in plants. Annu. Rev. Ecol. Syst. 27, 237–277.

McClay, A.S., Bourchier, R., Butts, R.A., Peschken, D.P., 2001. *Cirsium arvense* (L.) Scopoli, Canada thistle (Asteraceae). In: Mason, P. G., Huber, J.T. (eds.). Biological Control Programmes in Canada, CABI, Wallingford, UK, pp. 318–330.

Mitchell, C.E., Power, A., 2003. Release of invasive plants from fungal and viral pathogens. Nature 421, 625–627.

Moore, R.J., 1975. The biology of Canadian weeds. 13: *Cirsium arvense* (L.) Scop. Can. J. Plant Sci. 55, 1033–1048.

Nuzzo V., 1997. Element Stewardship Abstract for *Cirsium arvense*. The Nature Conservancy, Arlington, VA, USA.

Olsen, S.R., Cole, C.V., Watanabe, F.S., Dean, L.A., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circ. 939, 1–19.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller T.H., Keeney, D.R. (eds.) Methods of soil analysis: part 2. Chemical and microbiological properties. Agron. Monogr. 9, 2nd edit. ASA and SSSA, Madison, pp. 403–430.

Peschken, D.P., Wilkinson, A.T.S., 1981. Biocontrol of Canada thistle (*Cirsium arvense*): releases and effectiveness of *Ceutorhynchus litura* (F.) (Coleoptera: Curculionidae) in Canada. Can. Entomologist 113, 777–785.

Pritekel, C., Whittemore-Olson, A., Snow, N., Moore, J.C., 2006. Impacts from invasive plant species and their control on the plant community and belowground ecosystem at Rocky Mountain National Park, USA. Appl. Soil Ecol. 32, 132–141.

Pysek, P., Richardson, D.M., 2007. Traits associated with invasiveness in alien plants: Where do we stand? In: Nentwig, W. (ed.), Biological Invasions, Ecol. Stud. 193, Springer, Berlin-Heidelberg, pp. 97–126.

Rajaniemi, T.K., Reynolds, H.L., 2004. Root foraging for patchy resources in eight herbaceous plant species. Oecologia 141**,** 519–525.

Raabova, J., Münzbergova, Z., Fischer, M., 2007. Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*. Biol. Conserv. 139**,** 348–357.

Schroeder, D., 1980. The biological control of thistles. Biocontrol News and Information 1, 9–26.

S-PLUS 2000. Professional Edition for Windows, Release 2. MathSoft, Seattle, WA, USA.

Torchin, M.E., Lafferty, K.D., Kuris, A.M., 2002. Parasites and marine invasions. Parasitology 124, 137–151.

van Kleunen, M., Schmid, B., 2003. No evidence for an evolutionary increased competitive ability in an invasive plant. Ecology*,* 84**,** 2816–2823.

van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M., Fischer, M., 2010. Are invaders different? A conceptual framework of comparative approaches for assessing determinants of invasiveness. Ecol. Lett. 13, 947–958.

Vitousek, P.M., 1990. Biological invasions and ecosystem processes: towards an integration of population biology and ecosystem studies. Oikos, 57, 7–13.

Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A., Losos, E., 1998. Quantifying threats to imperiled species in the United States. Bioscience, 48, 607–615.

Williams, J.L., 2009. Flowering life-history strategies differ between the native and introduced ranges of a monocarpic perennial. Am. Natural. 174**,** 660–672.

Williams, J.L., Auge, H., Maron, J.L., 2008. Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens. Oecologia, 157**,** 239–248.

Williams, J.L., Auge, H., Maron, J.L., 2010. Testing hypotheses for exotic plant success: parallel experiments in the native and introduced ranges. Ecology, 91, 1355–1366.

Zou, J., Rogers, W.E., Siemann, E., 2007. Differences in morphological and physiological traits between native and invasive populations of *Sapium sebiferum*. Funct. Ecol. 21, 721–730.

Chapter 4

Table 1 Location and climate information of sample sites, wherefrom seeds for the experiment were collected.

Distribution range	Population	Latitude/longitude	Altitude (m.)	Climate
Native	Spain	N 41° 29' 40" E 1° 19' 27"	703	Short moderate winters, long, hot and dry summers, mediterranean continental climate
	Czech Republic	N 49° 51' 06" E 14° 12' 37"	460	Long, cold winters, short summers, continental climate.
Invasive	Nebraska	N 40 ° 54' 00" W 96 ° 23' 13"	338	Cold winters, long, hot summers, humid continental climate.
	Illinois	N 41° 19' 16" W 89° 55' 59"	237	Cold winters, long, hot summers, humid continental climate

Table 2 Chemical composition of the two substrates used in the experiment. The values are mean values from 10 samples ($p < 0.001$ for all tests), standard errors are shown.

Substrate	g. kg-1 Nitrogen	g. kg-1 Total soil Carbon	g. kg-1 Soil carbon in Carbonates	g. kg-1 Soil organic Carbon	Phosphorus (mg/1000g)
Poor	0.72 Mean +- SE 0.003	1.21 Mean +- SE 16.473	0.04 Mean +- SE 0.003	1.14 Mean +- SE 16.474	62.14 Mean +- SE < 0.001
Rich	0.88 Mean +- SE 0.003	8.42 Mean +- SE 0.022	1.11 Mean +- SE 0.007	7.31 Mean +- SE 0.023	2.46 Mean +- SE 2.722

Table 3. Factors determining aboveground growth of *Cirsium arvense*. R^2 values for significant factors are shown. *** $p < 0.001$, **$p < 0.01$, * $p < 0.05$, and. n.s. $p \geq 0.05$. N = 80. Flowerhead biomass with aboveground biomass as a covariate represents biomass allocation to flowerheads.

Dependent variable	Df	Flowerhead biomass	Flowerhead biomass (Aboveground biomass as covariate)	Aboveground biomass	No. of stems	Plant height
Range	1	n.s.	n.s.	0.014 *	n.s.	n.s.
Country	2	n.s.	n.s.	0.055 ***	0.054 ***	n.s.
Year	1	0.208 ***	0.226 ***	n.s.	0.423 ***	0.143 ***
Substrate	1	0.139 ***	0.086 ***	0.507 ***	0.149 ***	0.074 **
Range x Substrate	1	n.s.	n.s.	0.056 ***	0.017 *	n.s.
Country x Substrate	2	n.s.	n.s.	n.s.	0.018 *	n.s.
Range x Year	1	0.058 **	0.057 **	n.s.	n.s.	n.s.
Substrate x Year	1	0.085 ***	0.072 **	n.s.	0.102 ***	n.s.
Country x Year	2	0.04 *	n.s.	n.s.	n.s.	n.s.
Range x Substrate x Year	1	n.s.	n.s.	n.s.	n.s.	n.s.
Country x Substrate x Year	2	n.s.	n.s.	n.s.	n.s.	n.s.

Table 4. Factors determining biomass of various parts of *Cirsium arvense* in 2009. R^2 values for significant factors are shown. * means $p < 0.05$, ** means $p < 0.01$ and *** means $p < 0.001$. n.s. means $p \geq 0.05$. N = 80.

See separate file in horizontal format

Chapter 4

Figure Legends

Figure 1. Effect of distribution range and substrate (poor/rich) on aboveground biomass of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Figure 2. Effect of distribution range and substrate (poor/rich) on healthy root biomass of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Figure 3. Effect of substrate (poor/rich) and year on biomass allocation to flowerheads of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Figure 4. Effect of distribution range and year on plant height of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Figure 5. Effect of distribution range and substrate (poor/rich) on number of stems per plant, of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Figure 6. Effect of distribution range and substrate (poor/rich) on) total plant biomass (aboveground biomass + root biomass) of *Cirsium arvense*. For the test of significance see Table 3 and 4. The values are mean + SE.

Chapter 4

Figure 1)

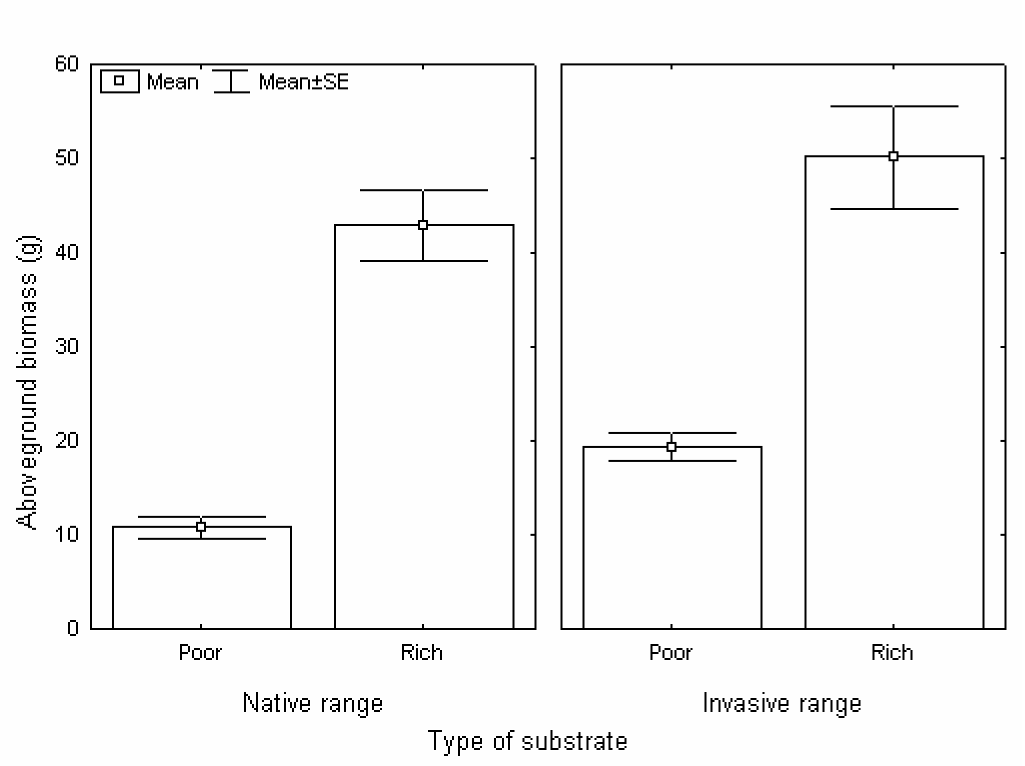
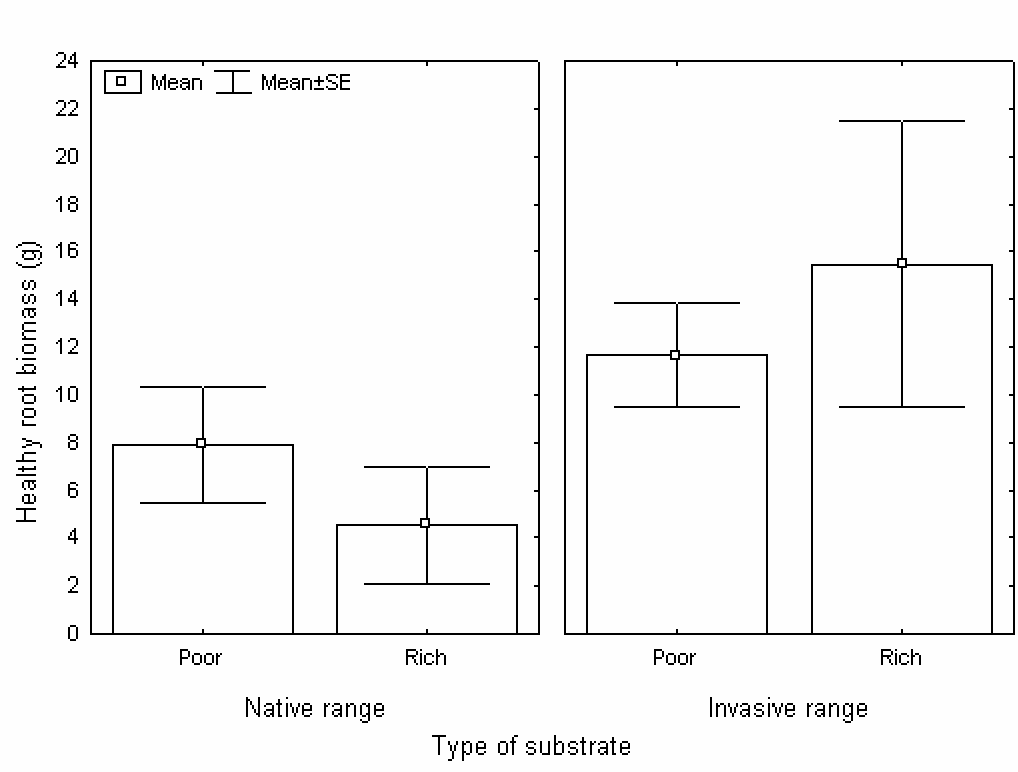
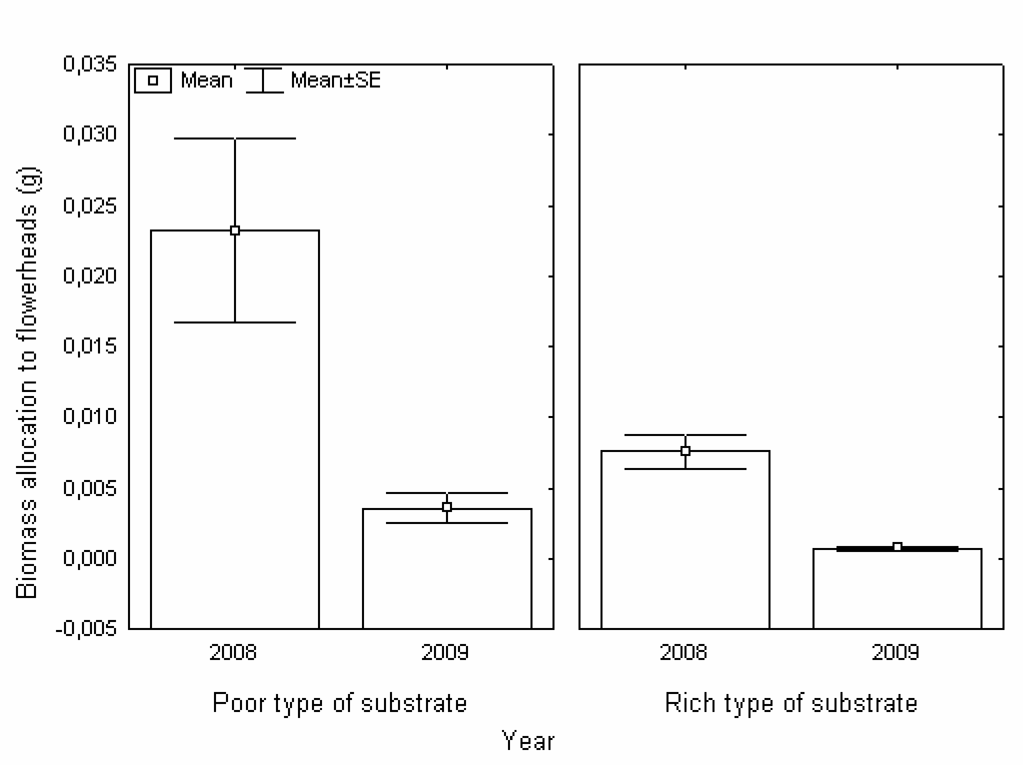


Figure 2)


Mean
Mean±SE
Healthy root biomass (g)
0
2
4
6
8
10
12
14
16
18
20
22
24
Poor
Rich
Poor
Rich
Native range
Invasive range
Type of substrate

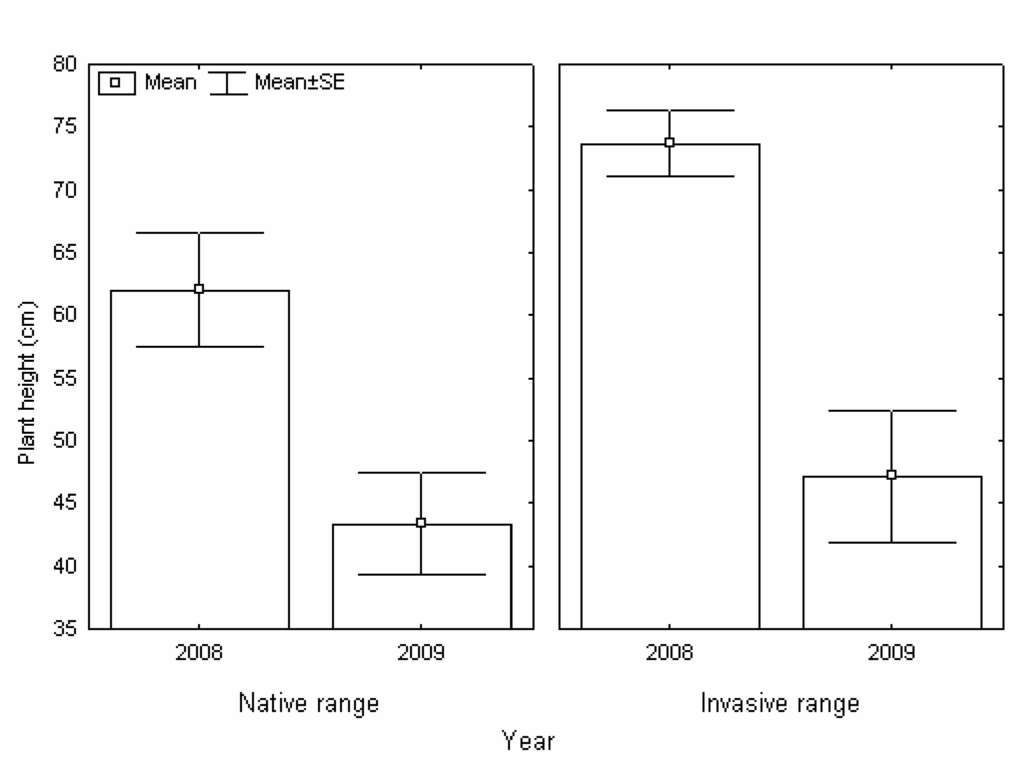
Chapter 4

Figure 3)



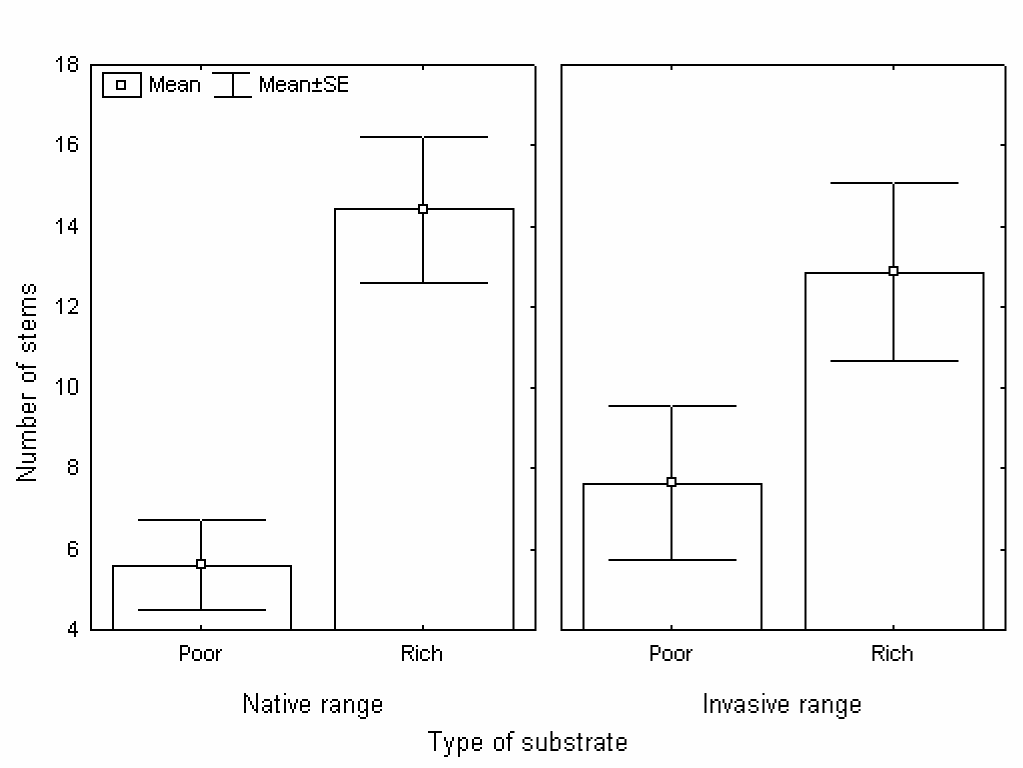
Chapter 4

Figure 4)



Chapter 4

Figure 5)



Chapter 4

Figure 6)

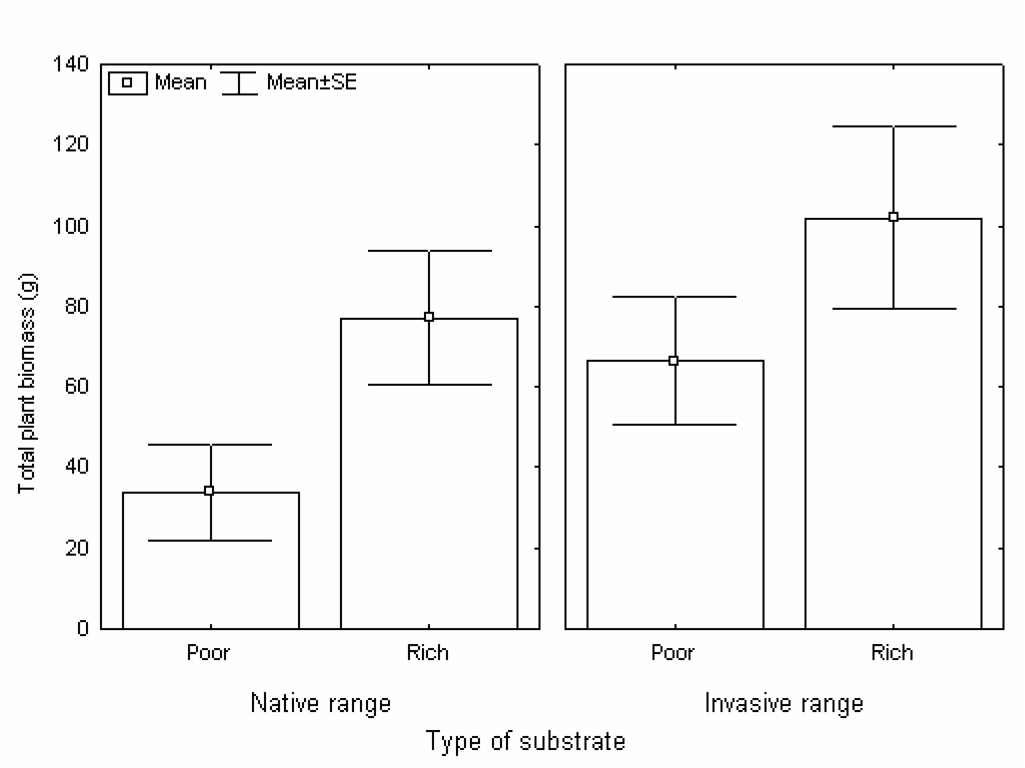


Table 4. Factors determining biomass of various parts of *Cirsium arvense* in 2009. R^2 values for significant factors are shown. * means $p < 0.05$, ** means $p < 0.01$ and *** means $p < 0.001$. n.s. means $p \geq 0.05$. N = 80.

Dependent variable	Df	Healthy root biomass	Damaged root biomass (Healthy + damaged root biomass as covariate)	Dead root biomass (Healthy + damaged + dead root biomass as covariate)	Root length	Root:shoot ratio	Total plant biomass
Range	1	0.098 **	n.s.	n.s.	n.s.	n.s.	n.s.
Country	2	0.122 *	0.151 ***	n.s.	n.s.	n.s.	0.133 *
Substrate	1	n.s.	0.061 *	0.095 **	0.068 *	0.245 ***	0.104 *
Range x Substrate	1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Country x Substrate	2	n.s.	0.091 *	n.s.	n.s.	n.s.	n.s.