Department of Ecology
Faculty of Science
Charles University in Prague

Inés Abela Hofbauerová

Effect of different natural enemies on performance of *Cirsium arvense*, Canada thistle, in the Czech Republic

Zuzana Münzbergová Prague, September 2007

Contents

I declare that this work was independently elaborated, only with the use of the cited references. I consent to lend it therewith all information will be properly cited.

3rd September 2007

Acknowledgments

My most sincere thank you is for my supervisor Zuzana Münzbergová, who guided me, taught me with patience, went with me to the field and revised this work so many times.

This thesis would not be possible without Svat'a Louda and Leland Russell, who are the original authors of this project in The University of Nebraska and revised my methodology at the beginning of the work.

For going with me to the field, thanks to Michal Kmet', Radka Krčmova, Ivana Franciskovic', Ján Libič, Marin Kundic', Robert Babic' Iguázel Pac, Hana Rychtrmocová, Ana Kladivová, Jozef Mitterpach and Zita Červenková. This trips to the field would not be possible without the finantial support of GAUK 43-203373 and 46-242171.

Thank you to Carmen Blubaugh and Jim Eckberg for language revision.

I also want to thank Eva Kozubíkova and other students of the department of Ecology. Also to my parents and my brother for being always by my side, even if they are far away, and also for going with me to the field.

Abstract

The aim of this project was to look at the effect of natural enemies on *Cirsium arvense* and to test the most effective herbivore hypothesis and the complementary herbivore hypothesis. We measured damages by different herbivore insect guilds and rust fungus *Puccinia punctiformis* in *Cirsium arvense* in six different populations in the surroundings of Prague (the Czech Republic). The populations were divided by humidity gradient. I assessed the spatiotemporal variability in damages by several natural enemies in *Cirsium arvense* between locally distinct populations with different abiotic conditions and seasons. In particular I was interested in identifying damage types that co-occur through space and time. I also wanted to discern whether the most considerable insect species have the biggest influence on the population dynamics of *C. arvense* (the most effective herbivore hypothesis) or if a combination of more species has more effect (complementary herbivore hypothesis).

Plant damage presence in *C. arvense* differ through seasons and are more common in wet site conditions. Damages that are co-occurring on the same individuals are necrosis at the top of ramets, eaten flower tops, cocoons in leaves, galls in stem and other stem damages. Other guilds of natural enemies that are co-occurring in the studied species are rust, holes and mines per leaf and froghoppers. The most important result is that guilds producing leaf necrosis were causing the same damage separately on *Cirsium arvense* as when combined with folivory guilds or with those which produce stem damage. This suggests looking for an insect from this guild could lead to a better biological control of *C. arvense* than to use a combination of various control insect species; nevertheless, there have to be more studies to confirm this fact.

Key words: *Cirsium arvense*, natural enemies, plant-insect interaction, plant damage, population dynamics.

1.Introduction

Invasive plants are the most important ecological results of the unprecedented alterations in the distribution of the earth's biota through human transport and commerce (Mack *et al.* 2000). The enemy releases hypothesis says 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, Carpenter, Cappuccino 2005). This can be due to several reasons: 1. Natural enemies regulate the size of the plant population, 2. These enemies have a greater effect on native species than on introduced species and 3. Plants are able to use the absence of enemies' regulation by having a bigger growth (Keane and Crawley 2002). The enemy releases hypothesis is the theoretical foundation of classical biological control (Hong *et al.* 2006), which is based on suppression of pest populations by natural enemies. Three types of biological control can be distinguished. In classical biological control humans introduce natural enemies to a new area and natural enemies are expected to establish viable populations there. In augmentative biological control natural enemies are repeatedly released to temporarily increase their density. In the conservation of natural enemies the key is in avoiding practices that are harmful to natural enemies that already live in the invasive weed range and implementing practices which benefit them (USA Office of technology assessment 1995).

Use of biological control might be one of the options to control plant invasions. There have been many attempts to use biological control to limit spread and density of invasive species (Delfosse 2000). The success of it, however, is not very high (Crawley 1989). Theoretical knowledge about effects of the herbivore insects on population dynamics of species is relatively limited and more information here may improve efforts to implement successful biocontrol programs (Dodd, 1940, Cofrancesco *et al.* 1998). One unresolved issue in biocontrol concerns whether the effect on plant population dynamics 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 1971, Huffaker, Messenger 1976, Murdoch *et al.* 1985, James *et al.* 1992). The validity of the abovementioned hypotheses has rarely been tested empirically. Simultaneously testing these two alternatives would provide a better knowledge on the effect of herbivore insects on plants, in general. Specifically, it would contribute to better planning of future biological control of invasive plants. There are, however, only few studies quantifying the impact of the combined vs. single effects of herbivore insects on plant population density (Juenger, Bergelson, 1998, Hufbauer, Root, 2002).

Results of Juenger and Gergelson (1998) revealed that the evolution of flowering phenology in scarlet gilia might be in 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. The limited number of such studies also shows that more studies on the additive and interactive effects of natural enemies on invasive plants are sorely needed. Studies of this type are missing not only in the invasive range but also in the native range of the species. Comprehensive tests of the single and combined effects of herbivore guilds on invasive plants in their native range are needed to better understand the interaction between natural enemies. Such information may help in designing effective biocontrol programs.

The aim of this study is to test these two alternative hypotheses for *Cirsium arvense*. *Cirsium arvense* (L.) Scop is one of the most problematic weeds in North America (Moore 1975) and a common native weed in Europe. In USA there is an attempt to manage its invasion by classical biological control (Schroeder 1980, Peschken 1984b, McClay *et al.* 2001). By measuring damage from various natural enemies of *C. arvense* and analyzing the interactions between the different natural enemies, I tested the most effective herbivore and complementary herbivore hypotheses. Specifically I tested if the effect of single herbivore guilds is independent or if the different herbivores interact with each other. I also wanted to see if the occurrence of damages caused by different natural enemies varied among populations with different abiotic conditions, localities and seasons. Finally I determined if and which types of damage co-occur under natural conditions and could thereby be concurrently used as biocontrol agents

The existing studies on *C. arvense* in the native range (Kruess A 2002, Eber, Brandl 2003, Kluth *et al.* 2002, 2003) rarely deal with interaction of several guilds of natural enemies. There is one study on the interactions between the rust fungus *Puccinia punctiformis* and ectophagous and endophagous insects from Germany (Kluth *et al.* 2001) and one study on mutualism between the shoot- base-boring weevil *Apion onopordi* and the rust fungus *Puccinia punctiformis* from Switzerland (Friedli, 2001). Examples of studies on the effect of herbivores on an invasive species in its native range using species related to *C. arvense* include a study from Germany exploring if the specialist enemies suppress *Carduus nutans* populations in its native range, and how this effect is related to other factors that may limit population growth (Jongejans *et al.* 2006).

These studies focused on the effect of one or two guilds on performance of the plant. A study that comprehensively examines the effect of more guilds on *C. arvense* and on their interaction is thus still needed.

2. Hypotheses

1) The effect of natural enemies on performance of *Cirsium arvense* is not independent i.e. the effect of one natural enemy is enhanced by the effect of a complementary guild of insects.

2) Occurrence of natural enemy damages and their effect on plant performance depend on habitat conditions, mainly moisture, of the sites.

3) Specific groups of natural enemies tend to co-occur more often together than expected by random.

3. Objectives

The objective of our project is to study the occurrence of different natural enemies in populations of *Cirsium arvense* differing in habitat conditions in the species native range and their effect on growth of the plant.

4.Material and Methods

4.1.Studied system

4.1.1. Plant species

The studied species is *Cirsium arvense* L. Scop. (Canada thistle, California thistle, creeping thistle, corn thistle, perennial thistle, field thistle). It is a herbaceous perennial of the aster family with erect stems 0.5 - 1.0 m tall, with prickly leaves, and an extensive creeping rootstock (Nuzzo 1997). It is a dioceous plant and it reproduces also by clonal shoots. In Europe grows in fields, glades, screes, empty places in sandbanks and in river willow-carrs from lowlands to mountain range (Slavík, Štěpánková 1998 - 2000). In the Czech Republic it is considered as an archeophyte (Pyšek *et al.* 2000). Despite its English name, Canada thistle 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).

4.1.2. Insect Community

Several guilds of natural enemies can be found in *Cirsium arvense*: leaf-feeding guild, root crown and stem-boring guilds, stem gall-forming guild, flower-head guild and rust fungus.

One of the main insects causing leaf damage in *C. arvense* is *Hadroplontus litura*. The species makes holes into the midribs on the underside of leaves. Females lay eggs into these feeding cavities. When the larvae hatch, they mine down the midrib into the stem (Peschken & Wilkinson 1981). *Cassida rubiginosa* (*Coleoptera: Chrysomelidae*) is also a leaf-feeder in *C. arvense*. This insect makes round punctures on the upper sides of *Cirsium arvense* leaves during its adult stage (Zwolfer 1966). The main insect causing stem-boring is *Apion onopordi* (*Coleoptera: Apionidae*)(Friedli, Bacher 2001). Insect causing stem galls is *Urophora car-*

dui (*Diptera: Tephritidae*). It has one generation per year and oviposits into vigorous shoots (Harris, Shorthouse, 1996). Insects causing seed-head damage are *Larinus planus (Coleoptera: Curculionidae*) and *Rhinocyllus conicus*. They oviposit into unopened flower head buds and the larvae feed on the developing florets, ovules and receptacle tissue (Louda & O'Brien 2002).

We also measured cover of rust fungus *Puccinia punctiformis*, a systematic disease which is present in the soil and is specific to *C. arvense* (Wandeler and Bacher 2006).

4.2. Characteristics of the studied area

Three different localities were selected in spring 2005. At each locality two sites were selected on a moisture gradient, one site is always next to a stream and the other is usually about 300 meters far away on a dryer place.

All sites were within 100 km from Prague (Table 1 for list of sites). These were in the surroundings of the villages Kytín, Milý and Výžerky (Annex A). The two sites in Kytín were 1 km from the village. The dry site was in a non-used meadow by a rural road. The wet site was by a brook which springs in Brdy Mountains. The two sites in Milý were 1 km from the village in Džbán Natural Park. The dry site was on the hill in a bushy place between a forest and a meadow. The wet site was by agricultural meadows and near Hřešický brook which springs in the Džbán Natural Park. The two sites in Výžerky were at the end of the village. The dry site was at the beginning of a small forest and the wet was by a brook.

Locality	Locality code	Latitude	Longitude	Altitude
Kytín wet	1	N 49º 51' 06"	E 14º 12' 37"	460 m
Kytín dry	2	N 49º 51' 04"	E 14º 12' 77"	440 m
Milý wet	3	N 50º 14' 10"	E 13º 51' 40"	310 m
Milý dry	4	N 50° 13' 19"	E 13° 51' 47"	335 m
Vyžerky wet	5	N 49° 56' 56"	E 14° 53' 95"	340 m
Vyžerky dry	6	N 49º 56' 72"	E 14º 53' 40"	358 m

Table 1. Geographic information of the *Cirsium arvense* sites.

4.3. Field design

Plots within sites were all situated within 100 meters. In each site 300 plants were selected (150 at Milý) in spring 2005. Plants in each site were marked within 10 experimental plots (5 in the case of Milý) with 4 sticks surrounded by a string. All plants per plot were marked, and there were about 30 plants in each plot. The plants were marked with a number with a plastic visible mark and one metal mark in the ground (for identifying it in case of loss of the plastic one).

4.4. Field measurements (measurements of plant performance and plant damage)

Each plant was measured in early, mid and late season. The early season measurements were made from ends of April to June in 2005 and April to May in 2006. We measured traits describing plant performance. These were root crown diameter measured with a digital caliper (push the caliper about 5 mm into the soil at a 45º angle around the base of the stem and the top of the root- moving aside all leave bases- and squeeze medium hard in order to get an estimate of the actual width of the juncture between root and stem, Louda unpubl.), number of leaves and length of the longest leaf of the marked plants.

The mid season measurements were made in July 2005 and 2006, in which we measured plant performance and plant damage by different natural enemies.

The data on plant performance measured in mid season included: Height of each plant, number of leaves and the length of the longest one in the vegetative ramets, number of flowering branches per plant and number of flowers in each 3rd flowering branch in the flowering ramets.

The measured damages were: Presence of cocoons and froghoppers per plant, % of folivory per plant, % leaf mines, presence of stem galls per plant (its length and width), floral head damage (estimated as the proportion of flower heads in the terminal inflorescence with external evidence of herbivory: necrosis, eaten, fallen floral head), % of rust per plant, % of stem damage, % of necrosis in leaves.

In these measurements we also recorded new seedlings and new vegetative ramets per plot.

The end season measurements were made in September 2005 and 2006. At this time we measured only the root crown diameter for second time in the season.

4.5. Additional information on the life cycle

In September to November 2005, seeds were collected from unmarked plants in the surroundings of the experimental plots in every site. Fifty viable seeds were sown in each of ten 50x50 cm plots per locality that were established in the neighborhood of the experimental plots. We also sowed seeds in plowed plots, to see whether the germination is higher in disturbed conditions New seedlings were searched for in the sowing plots as well as in adjacent control plots (plots without seed addition) in spring 2006.

4.6. Full life cycle of the species

I wanted to predict the growth of populations of *Cirsium arvense* in the populations in Kytín, Milý and Výžerky by constructing population matrices of *Cirsium arvense*. This part could not be done because I could not recognize which ramets from the 1st season corresponded to the ramets of the 2nd season in the experimental plots. This is due to the fact that *Cirsium arvense* is a clonal plant and shoots grow from the roots in different places around the mother plant every spring. Doing the matrices would be possible if I could have assign ramets from 1st season to ramets in the 2nd season.

4.7. Data analysis

The data from both seasons were analyzed with the programs Microsoft Excel 2003, Statistica 7.1., S-Plus 6.2. Professional and Canoco for Windows.

Plant damages were measured as % of damaged plant but I converted this measure to presence/absence of plant damage for data analysis.

Data from the sites in Milý were not included in analyses due to that the wet site in Milý was plowed the 2nd season and we could not thus compare the site conditions effect in this locality.

4. 7.1. Occurrence of damages

I calculated the mean of plant damages in every site I measured in the summer measurements in both seasons. This enabled me to have a first view of the more frequent damages and less appeared damages in the studied sites.

4. 7.2. Co-occurrence of all the measured damages

To analyze patterns of co-occurrence of the different plant damages measured in the summer measurements we used detrended correspondence analysis (DCA) in Canoco for Windows. Eleven natural enemy damages were measured. All the eleven plant damages were chosen "as species" and all our plants were samples. Rare species were downweighted in the analysis.

4. 7.3. Pair-wise co-occurrence of damages

To know whether one damage co-occurred with another more or less often than expected by random concurrence of each pair of damages was counted. Co-occurrence of damages was counted for 1st and 2nd season separately. These data were analyzed with Chi-square test in Statistica.

4. 7.4. Factors determining occurrence of the damages

4. 7.4.1. Factors determining occurrence of single natural enemies

The data on the occurrence of single types of plant damages caused by natural enemies were studied using logistic regression in S-PLUS. Three analyses of this kind were done.

First, we tested the effect of year, number of leaves per plant in spring, locality and site conditions for the two seasons combined. All second and third order interactions were also tested. We tested it with Chi square test.

We then analyzed with logistic regression how the single natural enemy damages depend on the number of leaves per plant in spring, locality and site conditions separately for each season. For the first year, 2005, the sites in Milý were excluded in the model because in the second season we had no data of the wet site in Milý due to that land was plowed in this site. The independent variables were the number of leaves in spring (a measure of plant size), locality and site conditions (wet, dry). The effect of number of leaves in interaction with locality and the effect of number of leaves in interaction with site conditions were also studied. We tested it with Chi square test.

4. 7.4.2.Factors determining occurrence of all plant damages together

To analyze the effect of year on occurrence of the different types of damage we used Canonical Correspondence Analysis (CCA) with blocks and unrestricted permutations within these blocks. As environment variables we choose the year. The covariables and also the blocks were the locality and the experimental plots were the plants were marked.

To estimate the effect of locality on occurrence of plant damage we used a CCA with blocks and unrestricted permutation within blocks where the environment data were the localities and the covariables and the blocks were year and site conditions.

To look at the effect of site conditions on plant damage, we used a CCA with blocks and unrestricted permutations within blocks. In this case the site conditions were chosen as environmental variables and the year and the localities where the covariables and the blocks. Rare species were down weighted in all multivariate analyses.

4.7.5. Test of the complementary herbivore hypothesis and most effective herbivore hypothesis

To analyze if the effect of various insect guilds on performance of *Cirsium arvense* is additive or interactive we used two step-wise regressions. In the one to test the additive effect of the damages the dependent variable was the stem base diameter at the end of the season and the independent variables were spring stem base diameter, locality, site conditions and the three most common damages which are number of holes per leaf, stem damage and leaf necrosis. We used only the three most common damages here, since the others were too few to yield sensible results.

In the stepwise regression to test the interactive effect of the damages the dependent variable was the stem base diameter, the independent variables were spring stem base diameter, locality, site conditions and the three most common damages. We included all 2-way interactions between the different types of damages into the model as well.

In both cases number of leaves in spring, site conditions and locality were included also in the base model. Stem base diameter at the end of the season was the dependent variable. In this way, the effect of the damages was tested after taking all the other factors into account.

To compare if one model was significantly better than the other we used compare models function in S-Plus. Distribution of stem base diameter data was normal.

5. Results

5.1. Occurrence of damages

The three most common plant damages in *Cirsium arvense* are holes per leaf, stem damage and leaf necrosis. Mines per leaf, eaten flowers, rust fungus, flower necrosis and eaten top are common damages. Stem galls and cocoons/leaf are less common damages. Froghoppers at the base of the root were rare damages (Table 2).

Plant damage	% damaged plants in 1st year						% damaged plants in 2nd year					
Site conditions	Wet			Dry			Wet			Dry		
Sites	1	3	5	2	4	6	1	3	5	2	4	6
Holes/ leaf	6,4	61,4	83,8	66,2	12,9	20,9	83,1	-	34,5	40,9	30,8	81,0
Stem damage	0,0	51,4	63,6	41,9	8,6	16,4	61,9	-	3,4	30,8	19,7	69,3
Leaf necrosis	0,0	60,0	75,7	14,9	6,5	12,7	16,1	-	83,9	4,4	10,3	54,6
Mines/ leaf	0,0	0,0	37,1	0,0	0,0	0,0	1,4	-	5,1	5,4	0,5	4,5
Eaten flower	0,0	2,9	26,8	29,7	0,5	1,5	0,8	-	23,2	6,3	0,0	2,5
Rust fungus	0,0	25,7	12,5	0,0	1,6	0,4	23,7	-	26,2	12,6	1,7	13,5
Flower necrosis	0,0	5,7	12,5	0,0	1,6	0,4	23,7	-	26,2	12,6	1,7	13,5
Eaten top	0,7	0,0	14,0	13,5	2,7	4,5	0,0	-	1,5	1,4	0,0	1,5
Stem galls	0,0	0,0	1,5	1,4	0,0	1,5	6,8	-	14,3	1,3	0,0	6,1
Cocoons/ leaf	0,0	0,0	2,9	2,7	0,0	1,1	12,7	-	11,9	2,5	4,3	5,5
Froghoopers	0,0	0,0	0,0	0,0	0,0	0,0	5,1	-	12,5	0,6	6,8	4,9

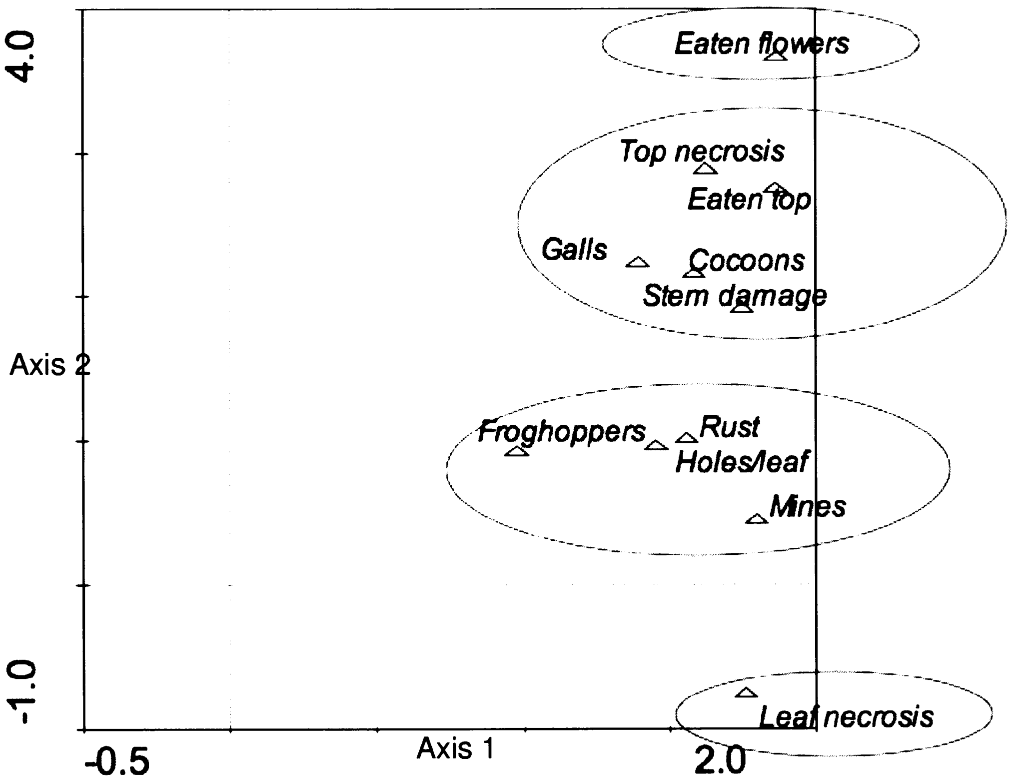
Table 2. Percent of damaged plants by all the measured natural enemies. – Means that we don't have data from the wet site in Milý because wet site was plowed the 2nd season.

5.2. Co-occurrence of all the measured damages

The damages that are co-occurring in *Cirsium arvense* are separated in four groups. Necrosis at the top of ramets, eaten flower tops, cocoons in leaves, galls in stem and other stem damages occur together in the same plant very often (Graph 1).

Other natural enemies that are co-occurring in the studied species are rust fungus, holes and mines per leaf and froghoppers (Graph 1).

Eaten flowers and leaf necrosis do not co-occur with other damages. 1st axis explains 37.1% of variability (Graph 1).



Graph 1. Co-occurrence of all the measured damages as determined using DCA analysis.

5.3. Pair-wise co-occurrence of damages

In general, there were more pair wise co-occurrences in the second season. The most common pair wise co-occurrence in the first measuring season, 2005, was leaf necrosis and folivory. Also the combination of folivory and stem damage was very common in plants of *Cirsium arvense* in the first season. These two double occurrences of plant damages were also the most common in the second measuring season (Table 3).

Other combinations of two damages occurring in more than 5% of the total of all the measured plants in the first measuring season were folivory and mines per leaf, leaf necrosis and mines per leaf, leaf necrosis and stem damage, stem damage and rust, stem damage and mines in leaves. Both in 1st and 2nd season leaf necrosis and rust were in 5% of the total plants. In the 1st measuring season there were more observed mines so the combinations of mines (windows) and other damages are more common in the 1st season than in the 2nd season (Table 3).

Leaf necrosis and cocoons per leaf; leaf necrosis and stem galls were common in the second season, in between 20% to 10% in the total of the measured plants (Table 3).

Double combinations of plant damages in the second season which were in between 10% and 5% of the total measured plants were:

Stem damage and eaten flowers, eaten flowers and folivory, stem damage and top necrosis, top necrosis and stem galls, top necrosis and cocoon, eaten top and folivory, eaten top and cocoon and leaf necrosis with rust. Only some of the less common pair wise co-occurrences in the second season were significant. Also the more common pair wise co-occurrence stem damage and top necrosis, in the second season, had a p=0,073, i.e. marginally significant (Table 3).

Double combination of plant damages	% of total plants 2nd season	Jaccard coefficient 2nd season	% of total plants 1st season	Jaccard coefficient 1st season
Folivory+Stem damage	23.5	0.279	11.60	0.233
Folivory+Leaf necrosis	12.4	0.198	14.20	0.331
Leaf necrosis+Cocoon	12.4	0.378	-	-
Leaf necrosis+Gall	10.6	0.346	-	-
Eaten flower+Stem damage	7.3	0.168	-	-
Eaten flower+Folivory	7.0	0.145	-	-
Stem damage+Top necrosis	5.9	**0.073**	1.9	0.093
Top necrosis+Gall	5.9	0.36	-	-
Top necrosis+Cocoon	5.6	0.341	-	-
Eaten top+Folivory	5.0	0.115	-	-
Eaten top+Cocoon	5.0	0.362	-	-
Leaf necrosis+Rust	5.0	0.176	5.0	0.176
Eaten top+Stem damage	4.9	1.147	3.3	0.136
Eaten top+Galls	4.9	0.36	-	-
Stem damage+Mines	4.0	0.106	5.1	0.189
Folivory+Mines	3.9	0.091	6.40	0.182
Eaten flowers+Top necrosis	2.8	0.153	-	-
Stem damage+Top necrosis	2.8	0.143	1.9	0.093
Leaf necrosis+Stem damage	2.8	0.191	5.70	0.145
Eaten flowers+Eaten top	2.7	0.168	-	-
Rust+Gall	2.7	0.289	-	-
Stem damage+Rust	3.1	0.078	5.4	0.192
Rust+Cocoon	2.6		-	-
Cocoon+Stem damage	2.5	**0.075**	-	-
Gall+Folivory	2.5	**0.065**	-	-
Eaten top+Folivory	2.1	**0.031**	-	-
Leaf necrosis+Eaten top	2.1	0.093	3.1	0.131
Leaf necrosis+Mines	1.7	**0.042**	6	0.203
Leaf necrosis+Eaten flower	1.5	**0.055**	-	-
Eaten top+Folivory	1.3	0.115	3.6	0.118
Leaf necrosis+Top necrosis	1.1	**0.041**	-	-
Rust+Top necrosis	1.1	**0.041**	-	-
Eaten top+Rust	0.9	0.092	-	-
Eaten flower+Rust	0.5	0.038	-	-

Table 3. Pair-wise co-occurrence of the different damages in marked plants of *Cirsium arvense* in the 1st and 2nd season. Significant values are marked in bold font.

5.4. Factors determining occurrence of all the damages

5.4.1. Factors determining occurrence of each single plant damage

We first tested the effect of the year, number of leaves per plant, site conditions, locality and their interaction with year on occurrence of the single plant damages (see Table 4). In this test presence of froghoppers depends on site conditions and year. In the 2^{nd} season and in wet sites this damage is more common.

All damages except rust depend on year. In the second season all the damages were more common. Cocoons and holes per leaf depend on the number of leaves. These damages are more common when plants have more leaves. Stem damage, eaten flowers and eaten top also depend on number of leaves per plant; when plants have less leaves, these three damages are more common (see Table 4).

All damages except froghoppers and rust depend on the locality. In the sites in Výžerky the damages are more common.

Stem galls, eaten flowers, eaten top, top necrosis, leaf necrosis and rust depend on the site conditions. In wet sites these damages are more common, leaf necrosis is more common in dry sites (see Table 4).

Damages	P-values	N° leaves	Locality	S. Condit.	Year	N° leaves * locality	S. Condit.* Locality/ Year	S. Condit. in locality * N° leaves	S. Condit. * Locality	Locality *Year	N° leaves * Year
Froghoppers	P-value	0.506	0.059	**0.045**	**<0.001**	0.997	0.999	0.998	0.993	0.984	0.998
	d.r.			-	+						
Cocoons	P-value	**0.007**	**0.044**	0.285	**<0.001**	0.190	0.239	0.349	0.264	0.821	0.283
	d.r.	+	+		+						
Hole/leaf	P-value	**<0.001**	**<0.001**	0.331	**<0.001**	**<0.001**	**<0.001**	0.956	0.191	0.974	0.878
	d.r.	+	+		+	-	+				
Mines	P-value	0.210	**<0.001**	0.287	**<0.001**	0.628	0.997	0.086	**<0.001**	0.989	0.999
	d.r.		-		+				+		
Galls	P-value	0.152	**<0.001**	**0.054**	**0,001**	**0.073**	0.083	0.519	0.318	0.880	0.631
	d.r.		-	-	-	-					
Stem damage	P-value	**<0.001**	**<0.001**	0.704	**<0.001**	**0.007**	**<0.001**	0.258	**0.098**	0.449	0.871
	d.r.	-	+		+	-	-		+		
Eaten flower	P-value	**<0.001**	**<0.001**	**0.080**	**<0.001**	**0.023**	**0.046**	0.052	0.018	0.237	0.822
	d.r.	-	-	-	+	-	+				
Eaten top	P-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.023**	**0.023**	0.180	0.505	0.711	0.524
	d.r.	-		-	+	-	+				
Top necrosis	P-value	0.088	**0.001**	**0.039**	**<0.001**	0.070	0.999	**0.002**	0.166	0.991	0.912
	d.r.		-	-	+			-			
Leaf necrosis	P-value	0.189	**<0.001**	**0.009**	**<0.001**	0.178	**<0.001**	0.104	0.124	0.536	0.322
	d.r.		+	+	-		-				
Rust	P-value	0.699	0.269	**0.035**	0.268	0.553	0.799	0.967	**<0.001**	0.915	0.737
	d.r.			-					-		

Table 4. Results of analyses testing the effect of number of leaves, locality, site conditions and year on occurrence of different plant damages. The table shows p-values and directions of the significant relationships. Significant values are in bold font. + means the direction of the relationships (d.r) is positive, - means it is negative.

Then we analyzed how the single natural enemy damages depend on the number of leaves per plant, locality and site conditions for each year separately. The presence of froghoppers per plant does not depend on the number of leaves of the plant in any year (Table 5).

Holes per leaf, stem damage, eaten flowers, eaten top and leaf necrosis depend on the number of leaves in the 1st season when including the site Milý in the model. When we exclude it, cocoons per leaf, stem damage, eaten flowers, eaten top and leaf necrosis depends on the number of leaves; when there are more leaves, plants have more of these damages (Table 5).

Stem damage, top and leaf necrosis and rust depend in the 1st season on the locality when including the site Milý in the model. In the first season stem damage, top and leaf necrosis and rust are more common in the sites in Výžerky. Holes per leaf, stem damage and leaf necrosis depend in the 1st season on the locality when excluding the site Milý from the model(Table 5).

The presence of holes per leaf, stem damage, leaf necrosis and rust in the1st season depend on the site conditions when including the site Milý in the model. This means that in wet sites in the 1st season there is normally more rust, stem damage, folivory and leaf necrosis in plants when we include site Milý in the model. When we exclude site Milý from the model the same damages depend on the 1st season on site conditions, and also eaten top (Table 5).

The presence of holes per leaf and eaten top depend on the 2nd season on the number of leaves; when the plant has more leaves in the 2nd season, there is higher probability that it has holes in leaf and eaten top.

The presence of mines per leaf, galls, stem damage, eaten flowers and leaf necrosis depend in the 2nd season on the locality. In the site Výžerky in the 2nd season these damages are more common (Table 5).

Presence of holes per leaf, mines, stem damage, leaf necrosis and rust depend in the 2nd season on the site conditions; in wet sites there is more probability that plants have more folivory, mines per leaf, stem damage, leaf necrosis and rust (Table 5).

We also analyzed the effect of the interactions between number of leaves and locality and number of leaves with site conditions: stem damage, holes per leaf and leaf necrosis depend on number of leaves in interaction with locality in the 1st season in the models where sites in Milý were included and excluded. That means that in plants in Výžerky with more leaves there is more stem damage but less necrosis. Eaten flowers depend in the 2nd season on number of leaves per plant in interaction with locality. This means that in the site Výžerky in plants with fewer leaves there are more eaten flowers. Holes per leaf depends in the 1st season on number of leaves in interaction with site conditions when excluding the sites in Milý. This means that in plants with more leaves in wet sites, plants have more holes per leaf. Stem galls, eaten flowers and leaf necrosis depends in the 2nd season on number of leaves in interaction with site conditions (Table 5).

Damages	P-values	N° leaves			Locality			s. Conditions			N° leaves/ Locality			N° leaves/ s.conditions		
		1st s. a)	1st s. b)	2nd s.	1st s. a)	1st s. b)	2nd s.	1st s. a)	1st s. b)	2nd s.	1st s. a)	1st s. b)	2nd s.	1st s. a)	1st s. b)	2nd s.
froghoppers	P-value	0.9	0.999	0.29	1	1	0.122	1	1	0.147	1	1	0.241	1	1	0.787
	d.r.															
cocoons	P-value	**0.03**	**0.045**	0.081	0.546	0.751	0.984	0.446	0.252	0.178	0.504	0.242	0.284	0.967	0.583	0.206
	d.r.	+														
hole/leaf	P-value	0.15	**<0.001**	0.008	0.914	**<0.001**	0.08	**<0.001**	**<0.001**	**<0.001**	**0.023**	**<0.001**	0.273	0.897	**<0.001**	0.613
	d.r.		-	+		+		+	+	+		+			+	
mines	P-value	0.9	0.999	0.535	1	1	**0.008**	1	1	**<0.001**	1	1	0.441	1	1	0.1
	d.r.						+			-						
galls	P-value	0.679	0.776	0.196	0.44	0.363	**0.018**	0.566	0.621	0.698	0.268	0.104	0.474	0.994	0.345	**0.027**
	d.r.						+									-
stem damage	P-value	**<0.001**	**<0.001**	0.06	**0.018**	**0.021**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.013**	**0.019**	0.489	0.927	0.998	0.08
	d.r.	+	+		+	+	+	-	+	+	+	-				
eaten flower	P-value	**<0.001**	**<0.001**	**0.048**	0.252	0.151	**<0.001**	0.77	0.485	0.698	0.988	0.944	**0.025**	0.484	0.471	**0.001**
	d.r.	+	-				+						-			+
eaten top	P-value	**<0.001**	**<0.001**	**0.001**	0.43	0.256	0.064	0.099	**0.035**	0.101	0.158	0.055	0.936	0.366	0.317	0.83
	d.r.	-	-	+					-							
top necrosis	P-value	0.143	0.999	**0.049**	**0.017**	1	0.868	0.959	1	0.143	0.999	1	0.117	0.995	1	0.375
	d.r.				-											
leaf necrosis	P-value	**<0.001**	**<0.001**	0.097	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.008**	**<0.001**	0.248	0.931	0.997	**0.014**
	d.r.	+	+		+	+	+	+	-	+	-	+				+
rust	P-value	0.27	0.504	0.46	**0.046**	0.306	0.062	**<0.001**	**0.106**	**<0.001**	0.208	0.104	0.691	0.483	0.106	0.505
	d.r.				-			-	-	+						

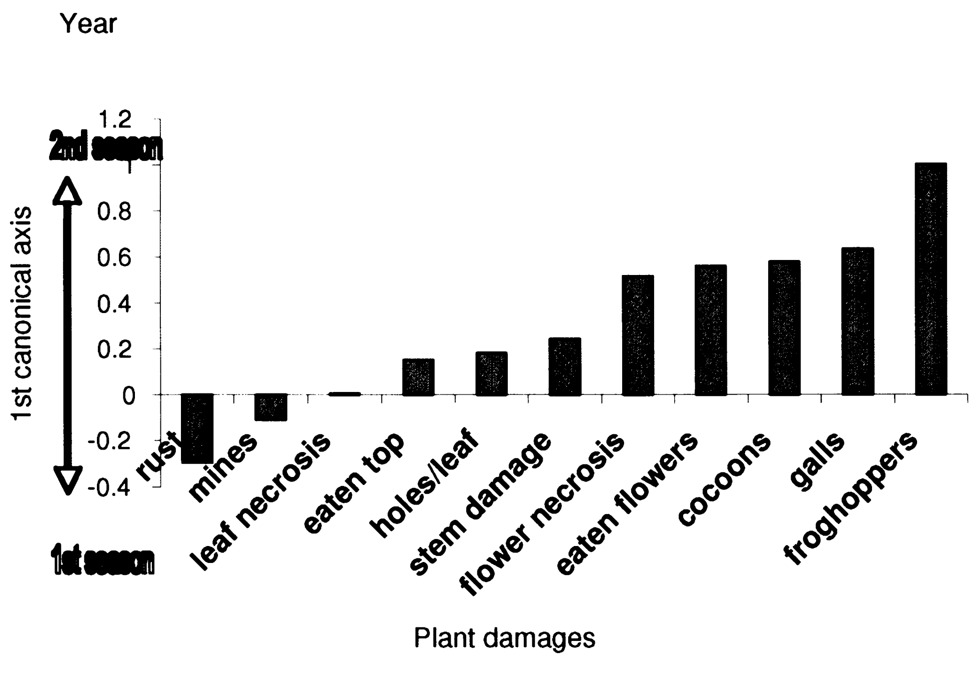
Table 5. Results of analyses testing the effect of number of leaves per plant, the locality and the site conditions on occurrence of different plant damages. The table shows p-values and directions of the significant relationships. Significant values are in bold font. + means the direction of the relationships (d.r) is positive, - means it is negative. 1st s. a) is the analysis including the wet and dry sites in Milý in the 1st season, 1st s. b) is the analysis without these sites in the 1st season and 2nd s. is the analysis in the second season.

5.4.2. Factors determining occurrence of all plant damages together

5.4.2.1. Effect of year

In the 2nd season there were more froghoppers, galls, cocoons, eaten flowers, flower and leaf necrosis, stem damage, holes per leaf and eaten top. Rust and mines seem to occur more often in the 1st season (Graph 2).

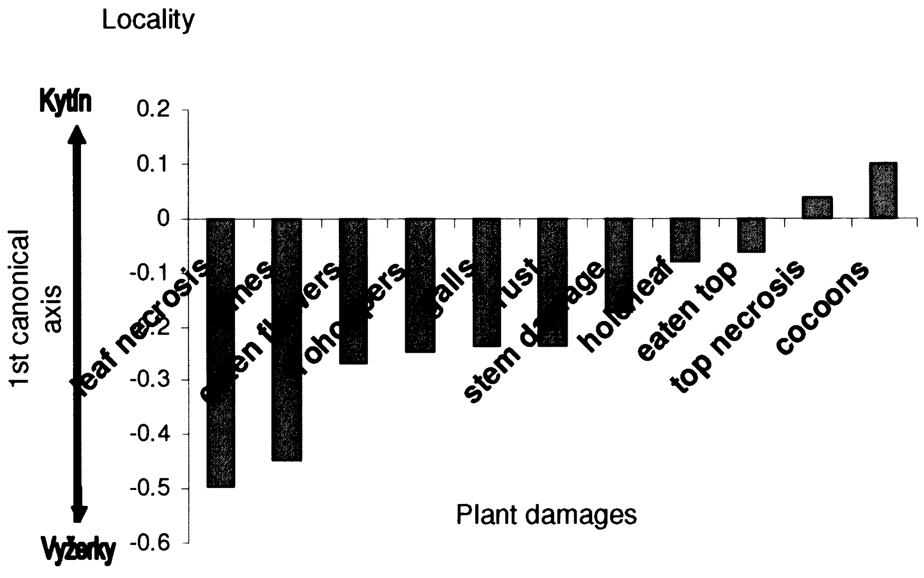
The % of variability explained by the year is 3.8. This is 12.2 % of the variation that can be explained by one ordination axis. The p value is 0.002.



Graph 2. Effect of the year on the plant damages as determined using CCA analysis. Negative values on the y-axis indicate affinity to the 1st season, positive to the 2nd season.

5.4.2.2. Effect of locality

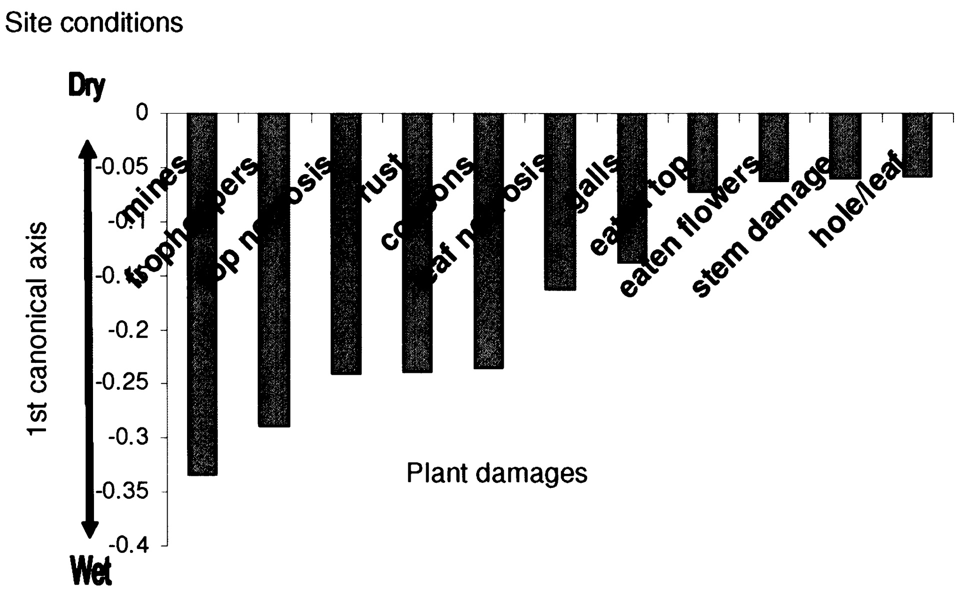
In the locality 1 (Kytín) the presence of cocoons and flower necrosis are more frequent than in locality 3. In the locality 3 (Výžerky) leaf damages as leaf necrosis, mines per leaf, rust fungus, holes per leaf and also other damages as eaten flowers, galls and stem damages are more frequent (Graph 3). The % of variability explained by the locality is 6.1. This is 19.7 % of the variation that can be explained by one ordination axis. The p value is 0.002.



Graph 3. Effect of the locality on the plant damage as determined using CCA analysis. Negative values on the y-axis indicate affinity to the locality Výžerky, positive to the locality Kytín.

5.4.2.3. Effect of site conditions

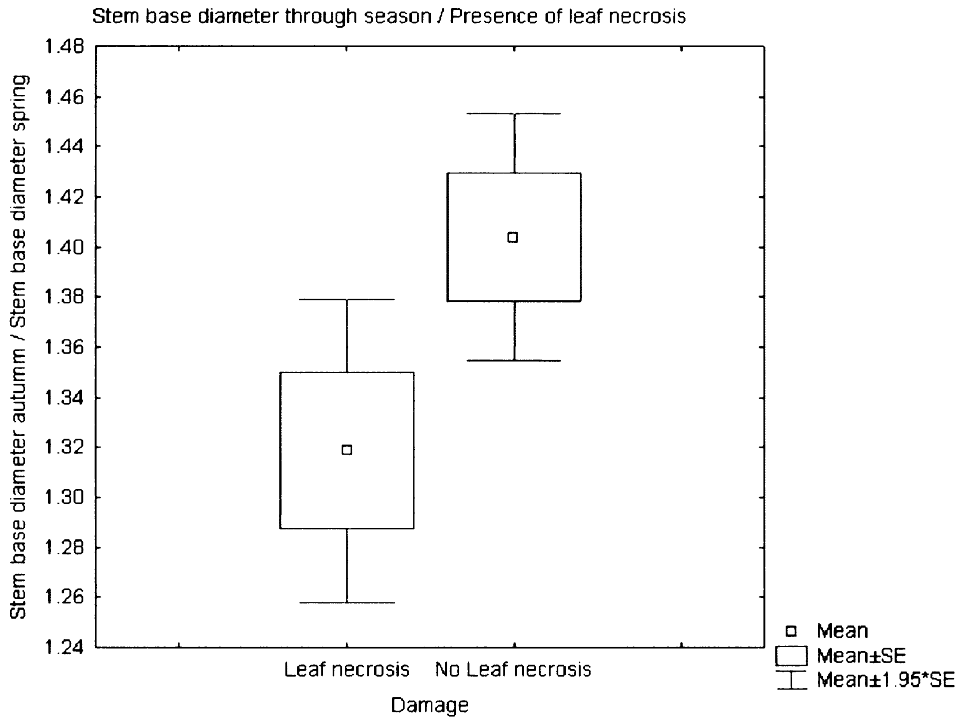
In the study of the effect of site conditions on the plant we observed that all the damages are more common in wet sites (Graph 4). The % of variability explained by the site conditions is 1.3. This is the 4.2 % of the variation that can be explained by one ordination axis. The p value is 0.002.



Graph 4. Effect of the site conditions on plant damage. Negative values on the y-axis indicate affinity to the wet sites, positive to the dry sites.

5.5. Additive and interactive effect of natural enemies on *Cirsium arvense*

In the analysis testing the dependence of change in stem base diameter of *Cirsium arvense* through year on season of measurement, locality and on the three more common damages we found out that the most significant damage is leaf necrosis. From the same analysis but with interactions between the three more common damages, which were folivory, stem damage and leaf necrosis, the damage chosen for the model was also leaf necrosis indicating no interactive effects between different damages (Graph 5). This suggests that the different insect guilds damaging *Cirsium arvense* do not affect each other.



Graph 5. Boxplot of plants of *Cirsium arvense* without leaf necrosis damage and with leaf necrosis damage (x axis). In the y axis difference of stem base diameter through season is showed.

5.6. Additional population information

In spring 2006 I found an average of 19.5 seedlings in wet sites in the sowing plots and 11.6 seedlings in average in dry sites. The germination of *Cirsium arvense* was higher in wet sites (Table 6).

Site	Number of seedlings in sowing plots and germination percentages							
	Plowed plots				Not plowed plots			
	Sown	Germination percentage	Control not sown	Germination percentage	Sown	Germination percentage	Control not sown	Germination percentage
Kytín wet	15	30%	0	0%	3	6%	0	0%
Kytín dry	10	20%	0	0%	2	4%	0	0%
Milý dry	6	12%	0	0%	2	4%	0	0%
Vyžerky wet	24	48%	0	0%	5	10%	0	0%
Vyžerky dry	19	38%	0	0%	3	6%	0	0%

Table 6. Seedlings which emerged in the second season from sowing plots in the studied sites and germination percentages.

I found new seedlings in the experimental plots in the 2nd season, 2006, in each locality, in which there were 300 plants marked, with the exception of Milý, where 150 plants were marked. The average of seedlings per site is 42.4. In the wet sites there were more new plants (Table 7).

Site	New seedlings/ site
Kytín wet	47
Kytín dry	30
Milý dry	31
Vyžerky wet	61
Vyžerky dry	43

Table 7. New seedlings per site in the 1st and 2nd season in the experimental sites

The average plant length in the marked plants was higher in wet sites in both seasons. We can then suppose that *Cirsium arvense* grows better in wet site conditions, by a water source, than in dry sites (Table 8).

Sites	Average plant lenght (cm)	
Seasons	1st season	2nd season
Kytín wet	57.80	76.80
Kytín dry	39.70	70.80
Milý wet	67.30	plowed
Milý dry	53.40	24.80
Vyžerky wet	48.30	67.50
Vyžerky dry	no data	64.30

Table 8. Average plant length in cm in *Cirsium arvense* in the studied sites

6. Discussion

6.1. Co-occurrence of all measured *Cirsium arvense* damages

Necrosis at the top of ramets and eaten flowers, which are co-occurring together in *Cirsium arvense* in the studied sites, could be caused by the same insect guilds since species as *Larinus planus* and *Rhinocyllus conicus* oviposits into flowers and causes flower heads to abort and thus to have necrosis (Louda & O'Brien 2002).

Urophora cardui, a gall-forming insect (Harris, Shorthouse, 1996), and *Apion onopordi*, a stem-boring insect (Friedli, Bacher 2001), cooccur in *Cirsium arvense* in the measured sites because stem galls and other stem damages co-occur together in *Cirsium arvense* in the studied sites. In the case of other stem damages it is difficult to predict which insects could be causing it because internal stem damage was not measured in this study.

The rust fungus *Puccinia punctiformis*, causing rust fungus, and *Hadroplontus litura* and *Cassida rubiginosa*, causing leaf damage, co-occur together. Our observations are similar to results of another study done in Germany by Kruess, 2002, of several insect guilds and rust fungus in *Cirsium arvense* which assents that the oligophagous chrysomelid beetle *Cassida rubiginosa,* from the leaf miner insect guild, is more likely to feed on leaf segments with rust fungus than on uninfected thistles. This co-occurrence may be explained because *Cirsium arvense* young shoots are infected by *Puccinia punctiformis* at the beginning of the season and leaves infected later could be more vulnerable to the attack of leaf eaters and leaf-miner insect guilds.

6.2. Effect of number of leaves per plant, locality and the site conditions on occurrence of measured *Cirsium arvense* damages

The effects of site conditions, number of leaves per plant and year were analyzed on the insect plant damage.

Plants in wet sites in Kytín and Vyžerky are taller than in dry sites. Also in the wet site in Milý in the 1st season plants are taller.

More damage by insects and rust was observed in wet localities, where the plants are taller. It can thus be expected that the overall effect of all these types of damages on plant performance is actually quite limited. There were more herbivore insect damages in plants in wet sites. Another study, Wool 2002, where galls abundance was counted in *Pistacia palestina* trees, had also higher levels of galls in wet years, which can be comparable with our gradient in wet sites (near a water stream).

This result is also consistent with the study of Coob *et al.* (1997), who found out that the herbivore damage caused by the stem- and cone-boring moth *Dioryctia albovitella* on *Pinus edulis* was six times greater in the most watered site they had compared with sites with less water.

Insect guild damages vary between seasons and sites, plants in site in Vyzerky had the most observed types of damage.

The insect guild damage which had the greatest effect on plant performance (stem base diameter throughout the measuring season) in *Cirsium arvense* was leaf necrosis. Leaf necrosis, the most common damage in *Cirsium arvense,* is not co-occurring often with other damages.

Folivory (Kruess 2002, Kluth, Kruess, Tscharntke, 2001,), stem and gall damage (Gange and Nice 1997, Bacher S, Schwab, 2000, Kluth, Kruess, Tscharntke, 2001, Eber and Brandl, 2003) an flower-head damage (Zwolfer, 1966, Louda, Potvin, 1995, Louda, Charles, O'Brien, 2002) are mentioned in the literature, but it is more difficult to compare results from our leaf necrosis data due to lack of literature.

Some studies have been performed examining the relation between plant size and insect damage as the one done by Eber and Brand, 2003. They observed that the number of *Urophora cardui* increases when the size of a *Cirsium arvense* decreases. In our study number of leaves per plant had no significant effect on the number of galls per plant.

The average infestation (% stem attacked) by all phytophaga was of 68% in *Cirsium arvense* (Freese 1993) in a study in Bavaria, and 12 insects species were associated with *Cirsium arvense*. This result was lower than in other thistle species (*C.vulgare, C.eriophorum, C.nutans, C.acanthoides, C. palustre* and *C. oleraceum*) and there was a bigger temporal niche separation as the attack rates and individual per plant were the lowest comparing with the other species from the study. This could be due to the small size and low apparancy of the plant species (Feeny 1976). This shows that *Cirsium arvense* is more resistant to attack of insects than other thistles. The % of plant damages in our marked *Cirsium arvense* plants reached 84%. It is difficult to compare % of plant damage

6.3. Additive and interactive effect of natural enemies on *Cirsium arvense*

One of the hypotheses I had was that multiple insect guilds will have a greater effect on the performance of *Cirsium arvense* than each of the guilds separately. I found out that the effect of the insect guild that produces leaf necrosis has the same effect alone as it does in combination with two other common guilds, stem borers and folivors. This suggests, that we could then use only one kind of guild for a biological control of *Cirsium arvense* and would not need to use a combination, which is more difficult to put in practice.

We should then look for an insect from this guild for a better biological control rather than to use a combination of various control species. Since I found out that one specific species has the greatest effect on *Cirsium arvense* performance I can say there is no need to combine it with other herbivore guild for an effective better biological control. Still, I have not examined how insect damages affect the seed recruitment and if damages caused by insects and rust fungus actually slow the individual growth over multiple field seasons and population growth, which is crucial for predicting the long term effects of the insects on plant population growth.

It is still not very clear whether a combination of species or one effective herbivore have the greatest effect on the plant population dynamics. James and McEvoy, 1992, support the strategy of introducing, as biological control, two complementary species which reduce *Senecio jacobea* in its invasive range in different stages and different times.

Junger and Bergelson, 1998, found out that the effect of three types of herbivores on Scarlet gilia is both additive and complementary.

Kluth *et al.*, 2001, found out that *Apion* species are more abundant in *Cirsium arvense* plants that are infected with the rust *Puccinia punctiformis.* The insects *Melanagromyza aeneoventris, Urophora cardui, Cassida rubiginosa* and some leaf miners preferred non infected plants. But the presence of rust did not reduce the presence of herbivores in my study. I found that this species of rust is not very abundant in *Cirsium arvense* in my sites in the Czech Republic and don't have a very significant effect. Thus, I can say that other natural enemies, which provoke leaf necrosis and leaf holes, have a more important impact than *Puccinia punctiformis* and therefore these have to be more accurately studied in the future.

There is still much debate in the field over whether the optimal effect on the population dynamics of plants is maximized using the most important herbivore (most effective herbivore hypothesis) (Turnbull, Chant 1961, Kakehashi *et al.* 1984, McEvoy *et al.* 1993) or the effect is bigger when more herbivore species are combined (complementary herbivore hypothesis) (Huffaker 1971, Huffaker, Messenger 1976, Murdoch *et al.* 1985, James *et al.* 1992). Results of studies proving the most effective herbivore hypothesis and the complementary species theory are different in different studies in *Cirsium arvense.*

I observed and analyzed the damages caused by herbivore guilds but since I did not study, observe or recollect the insects on *Cirsium arvense* I could not connect the plant damages with a specific insect. Since some of these insects, as *Cassida rubiginosa* (Bacher,Luder, 2005, Kluth 2001, 2002, 2003) and *Rhinocyllus conicus* (Louda 1998, Dodge 2005) are frequently studied because of their uses in biological control, it would have been better to do further analysis of insect presence in this study.

6.4. Additional population information

In the sowing plots the maximum germination percentage was 38%, in the dry site in Vyžerky in plowed plots, and the minimum was 4%

in the dry site in Milý in not plowed plots. This suggests *Cirsium arvense* has higher germination rates in disturbed conditions.

This study is based on a field experiment. This is better than a greenhouse experiment because we can see how plants grow naturally and can see a wide range of damages that maybe we could not see if plants were planted in a greenhouse, where not all insects or rust could have damaged the plants. Also, we could observe plants on sites with different habitat conditions. Instead, a greenhouse experiment would give more standard conditions and we would be able to recognize all plants through the second season, which is more problematic in the field, since *Cirsium arvense* is a clonal plant and new shoots in the second year can grow in some other place than the first year. Also the effect of water resource would be better by adding or restricting watering.

7. Conclusion

The most common natural enemy damages found in *Cirsium arvense* in the studied sites were folivory, external stem damage and leaf necrosis.

The most common damages were not co-occurring together. Folivory was co-occurring with leaf mines and with rust caused by *Puccinia punctiformis*. External stem damage was co-occurring with stem galls and top necrosis, eaten top and leaf cocoons. Leaf necrosis and eaten plant tops were not often co-occurring with other damage.

Insect guild damages and damage caused by *Puccinia punctiformis* were more common in sites which were near water streams, which shows *Cirsium arvense* tends to be more damaged in wet rather than in dry conditions.

Our results don't support the complementary species theory because I found the effect of the most common damage, leaf necrosis, on stem base diameter difference through a measuring season is the same when damaging alone and when damaging with folivory or stem damage, the two more common plant damages in *Cirsium arvense*.

After studying insect damages in plants I concluded that when looking at insect guild damages of a plant there is a need to identity which insect species causes the damage for a better understanding and analysis of the plant damages and insect-plant interactions.

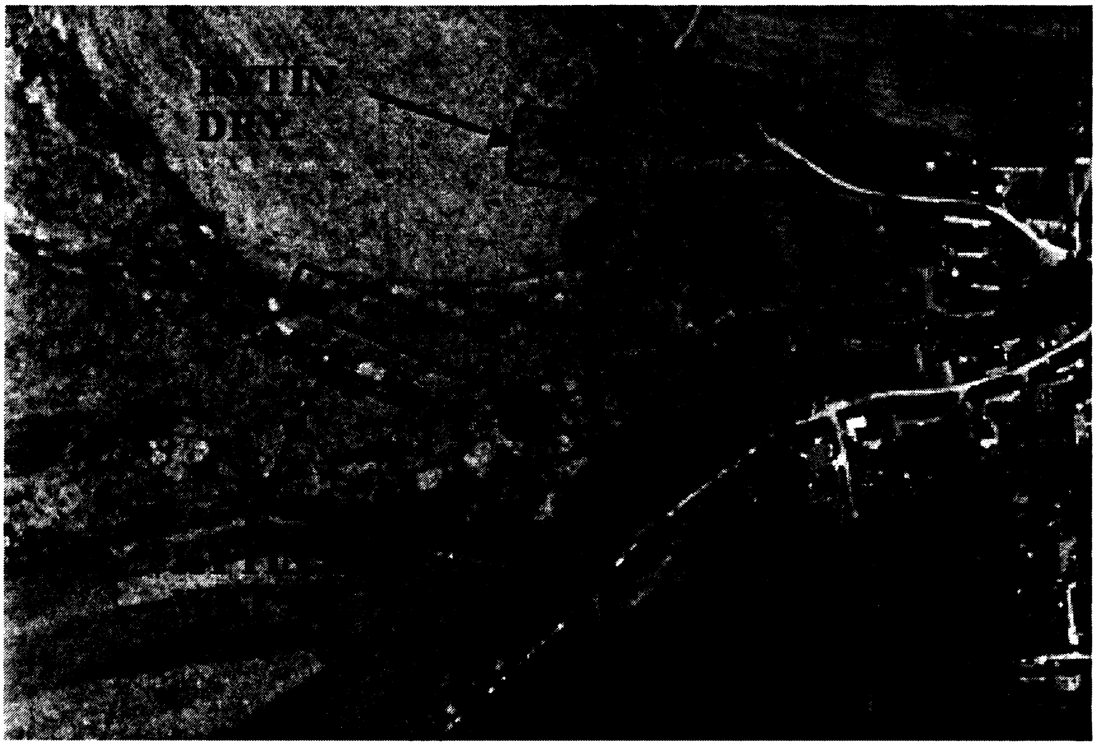
The study of particular insects and the connection between plant damage and insect in *Cirsium arvense* is needed for a better understanding of the system and the effect of the insect damages in the plant populations.

This is one of the more complete studies looking at the effect of a wide range of insect guilds damages on the performance of *Cirsium arvense* in its native range.

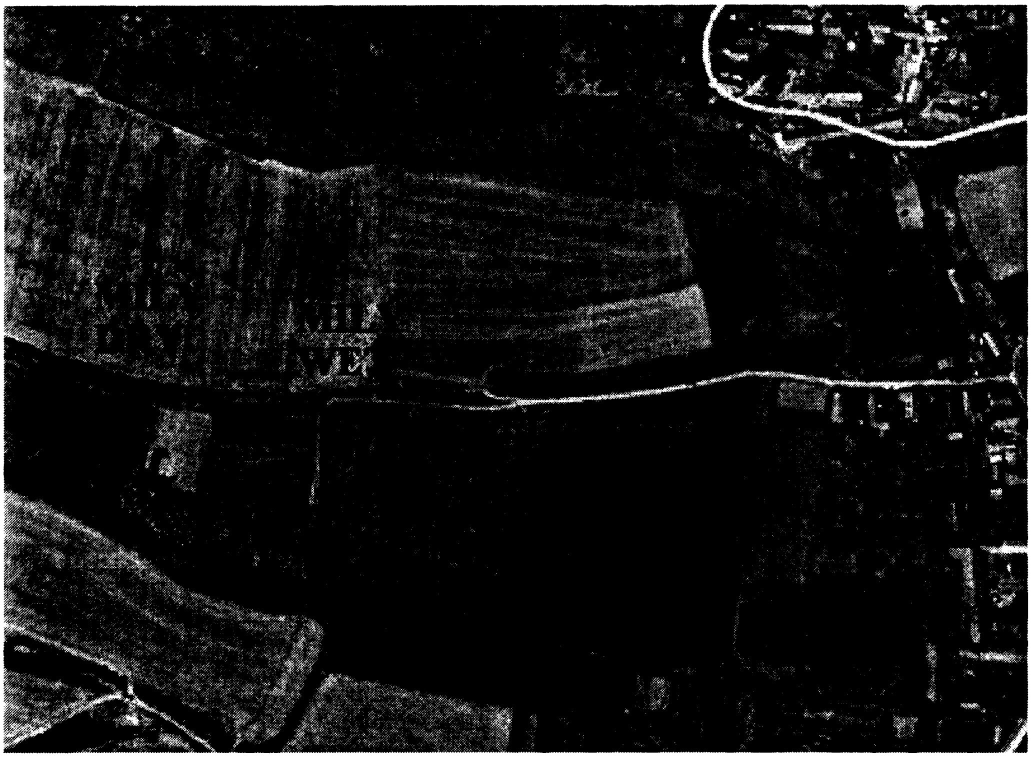
8. Annexes

Annex A. Mapped locations of study sites.

Map 1. Sites at Kytín



Map 2. Sites at Milý



Map 3. Sites at Výžerky

Annex B. Primary data. Means of plant damages (froghoppers, galls per plant, % of leaf necrosis per plant, cocoons per leaf, mines per plant, holes per plant, galls per plant, volume and height of the galls, stem damage, eaten flowers per plant, eaten tops per plant, top necrosis and % rust per plant) and performance (stem base, seedlings per plot, number of flowers per plant and number of leaves per plant) of the 1st and 2nd season measurements

Site	Stem base diameter spring 1st season mean	Number of leaves 1st season mean	Spring length leaf 1st season mean	Number of flowers/plant mean 1st season	Summer new ramets/site 1st season mean
Kytín wet	4.497	9.956	7.494	7.800	0.000
Kytín dry	3.499	7.221	8.643	1.865	0.029
Milý wet				2.447	0.000
Milý dry	2.794	6.863	6.614	0.855	0.015
Vyžerky wet	4.317	5.534	0.000	10.327	0.007
Vžerky dry	4.387	7.569	11.372	5.775	0.082
Total mean	3.939	7.210	8.344	4.924	0.031

Site	Spring new seedl./site 1st season mean	Summer new seedl./site 1st season mean	Galls presence 1st season mean	Froghopper presence 1st season mean	% Rust 1st season mean
Kytín wet	0.059	0.000	0.115384615	0	0.000
Kytín dry	0.004	0.106	0.057692308	0	1.882
Milý wet	0.000	0.000	0.067669173	0	0.284
Milý dry	0.000	0.014	0.094637224	0	0.037
Vyžerky wet	0.067	0.043	0.059405941	0	6.021
Vžerky dry	0.000	0.000	0.079957356	0	0.276
Total mean	0.019	0.037		0	2.027

Site	% Cocoons/leaf 1st season mean	% Mines 1st season mean	% Holes/leaf 1st season mean	Stem gall presence 1st season mean	Stem gall length 1st season mean
Kytín wet	0	0	7.143	0	0
Kytín dry	0.0110	0	5.037	0.004	0.007
Milý wet	0	0	10.975	0	0
Milý dry	0	0	5.074	0	0
Vyžerky wet	0.0458	3.269	11.810	0.0141	0.0176
Vžerky dry	0.0103	0	4.284	0.0172	0.0172
Total mean	0.0164	0.799	7.301	0.0086	0.0104

Site	% Stem damage 1st season mean	Presence eaten flowers 1st season mean	Presence eaten top 1st season mean	Presence top necrosis 1st season mean	% leaf necrosis 1st season mean
Kytín wet	0	0.057	0.029	0	0
Kytín dry	2.169	0.015	0.037	0	0.680
Milý wet	7.057	0.014	0.028	0.035	5.390
Milý dry	3.235	0.007	0.037	0.022	2.444
Vyžerky wet	9.433	0.074	0.138	0.159	12.261
Vžerky dry	2.414	0.042	0.041	0.003	2.638
Total mean	4.658	0.036	0.061	0.047	4.773

Site	Galls presence 2nd season mean	Gall width 2nd season mean	Gall length 2nd season mean	Total of flowers per plant 2nd season mean	Eaten flower presence 2nd season mean
Kytín wet	0.054	2	1.333	3.372	0.147
Kytín dry	0.013	2.333	1	2.378	0.006
Milý wet					
Milý dry	0	0	0	0.466	0.015
Vyžerky wet	0.098	1.696	1.0304	3.991	0.192
Vžerky dry	0.050	1.778	1	2.168	0.178
Total mean	0.053	1.762	1.02	2.743	0.127

Site	Eaten top presence 2nd season mean	% of leaf necrosis 2nd season mean	% Rust 2nd season mean	Top necrosis presence 2nd season mean	% Stem damage 2nd season mean
Kytín wet	0.147	4.814	0	0.209	14.192
Kytín dry	0.064	1.032	2.019	0.128	4.038
Milý wet					
Milý dry	0.0150	3.1579	0	0.015	4.962
Vyžerky wet	0.1041	14.4006	4.700	0.136	11.435
Vžerky dry	0.0990	13.4158	0.842	0.109	22.946
Total mean	0.0896	9.0470	2.108	0.122	

Site	Eaten top presence 2nd season mean	% of leaf necrosis 2nd season mean	% Rust 2nd season mean	Top necrosis presence 2nd season mean	% Stem damage 2nd season mean
Kytín wet	0.147	4.814	0	0.209	14.192
Kytín dry	0.064	1.032	2.019	0.128	4.038
Milý wet					
Milý dry	0.0150	3.1579	0	0.015	4.962
Vyžerky wet	0.1041	14.4006	4.700	0.136	11.435
Vžerky dry	0.0990	13.4158	0.842	0.109	22.946
Total mean	0.0896	9.0470	2.108	0.122	

Site number	% Holes/leaf 2nd season mean	% Stem damage 2nd season mean	Mines presence 2nd season mean
1	21.462	14.192	5.433
2	5.827	4.038	0.609
3			
4	9.098	4.962	1.880
5	15.595	11.435	1.855
6	21.436	22.946	

9. References

Bacher S, Schwab F, 2000, Effect of herbivore density, timing of attack, and plant community on performance of creeping thistle *Cerium ravens* (L.) Scoop. (*Asteraceae*), Biocontrol Science and Technology, Vol.10, Issue 3, pp.343–352

Bacher S, Luder S, 2005, Picky predators and the function of the faecal shield of a cassidine larva, Functional Ecology, Vol. 19, Issue 2, pp. 263-272

Cappuccino N, Carpenter D, 2005, Invasive exotic plants suffer less herbivory than non-invasive exotic plants, Biological Letters, Vol.1, Issue 4, pp.435–438

Coob 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, Vol. 109, Issue 3, pp. 389-397

Cofrancesco AF, 1998, Overview and Future Direction of Biological Control Technology, Journal of Aquatic Plant Management, Vol.36, pp.49-53

Colautii RI, Ricciardi A, Grigorovich IA, MacIsaac HJ, 2004, Is invasion success explained by the enemy release hypothesis?, Biological Letters, Vol.7, pp.721–733

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

Delfosse ES, 2000, Biological control: important tool for managing invasive species, Agricultural Research, Vol.48, p.2

Dodd AP, 1940, The biological campaign against prickly-pear, Commonwealth Prickly-pear Board Bulletin, Brisbane, Australia, p.177

Dodge GJ, 2005, Ecological Effects of the Biocontrol Insects*, Larinus Planus* and *Rhinocyllus Conicus*, on Native Thistles (dissertation), Digital Repository at the University of Maryland, available at: https://drum.umd.edu/dspace/handle/1903/2413?mode=simple

Friedli J, Bacher S, 2001, Mutualistic interaction between a weevil and a rust fungus, two parasites of the weed *Cirsium arvense*, Oecologia, Vol.129, Issue 4, pp.571-576.

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, Vol.22, pp.219 –226

Gange AC, Nice HE, 1997, Performance of the thistle gall fly, *Urophora cardui*, in relation to host plant nitrogen and mycorrhizal colonization, New phytologist, Vol.137, Issue 2, pp.335-343

Harris P, Shorthouse JD, 1996, effectiveness of gall inducers in weed biological control, Canadian Entomologist, Vol. 128, pp. 1021-1055

Hong L, Stiling P, Pemberton RW, Peña J, 2006, Insect herbivore faunal diversity among invasive, non-invasive and native *Eugenia* species: Implications for the enemy release hypothesis, Florida Entomologist, Vol.89, Issue 4, pp.475–484

Huffaker C, 1971, Biological control, Plenum Press New York (book)

Huffaker C, Messenger PS, 1976, Theory and practice of biological control, Academic Press (book).

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, Vol.147, Part 2, pp.204–213

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

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- Jacobaeae*) – An experimental analysis, Journal of Applied Ecology, Vol.29, Issue 3, pp.589-596

Jongejans E *et al.*, 2006, What controls the population dynamics of the invasive thistle *Carduus nutans* in its native range?, *Journal of Applied Ecology,* Vol.43, Issue 5, pp.877-886

Juenger T, Bergelson J, 1998, Pairwise versus diffuse natural selection and the multiple herbivores of Scarlet gilia, *Ipomopsis aggregata*, Evolution, Vol.52, Issue 6, pp.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, The Journal of Applied Ecology, Vol.21, Issue 1, pp.115-131

Keane RM, Crawley MJ, 2002, Exotic plant invasions and the enemy release hypothesis, Trends in Ecology and Evolution, Vol.17, Issue 4, pp.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, Vol.38, pp.548–556

Kluth S, Kruess A, Tscharntke T, 2002, Insects as vectors of plant pathogens: mutualistic and antagonistic interactions, Oecologia, Vol. 133, Issue 2, pp. 548–556

Kruess A, 2002, Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*, Oecologia, Vol. 130, Issue 4, pp. 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, Vol. 40, Issue 2, pp. 334-343

Kolar C, Lodge DM, 2001, Progress in invasion biology: predicting invaders, Trends in Ecology and Evolution, Vol. 16, Issue 4, pp. 199-204

Louda SM, 1998, Population growth of *Rhinocyllus conicus (Coleoptera: Curculionidae*) on two species of native thistles in prairie, Environmental Entomology, Vol. 27, Issue 4, pp. 834-841

Louda SM, Potvin MA, 1995, Effect of inflorescence-feeding insects on the demography and lifetime fitness of a native plant, Ecology,Vol. 76, Issue 1, pp. 229-245

Louda SM, Charles W, O'Brien, 2002, ,Unexpected Ecological Effects of Distributing the Exotic Weevil, *Larinus planus* (F.), for the Biological Control of Canada Thistle, Conservation Biology, Vol. 16, Issue 3, pp. 717–727

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA, 2000, Biotic Invasions: Causes, Epidemiology, Global Consequences, and Control, Ecological Applications, Vol.10, Issue 3, pp.689-710

McClay AS, Bourchier R, Butts RA, Peschken DP, 2001, *Cirsium arvense* (L.) Scopoli, Canada thistle (*Asteraceae*), pp.318-330. In Mason PG, Huber JT (eds.), 2001, Biological Control, Programmes in Canada, 1981-2000, CABI Publishing, Wallingford, United Kingdom

McClay AS, Chaper 17, Canada thistle, In: Van Driesche R *et al.*, 2002, Biological Control of Invasive Plants in the Eastern United States, USDA Forest Service Publication FHTET-2002-04, 413 p.216-228

McEvoy PB, Rudd NT, Cox CS, Huso M, 1993, Disturbance, competition, and herbivory effects on ragwort *Senecio Jacobaea* populations, Ecological Monographs, Vol.63, Issue 1, pp.55-75

Mitchell CE, Power AG, 2003, Release of invasive plants from fungal and viral pathogens, Nature, Vol.421, pp.625–627

Moore RJ, 1975, Biology of Canadian weed *Cirsium arvense* (L) Scop, Canadian Journal of Plant Science, Vol.55, Issue 4, pp.1033-1048

Murdoch WW, Chesson J, Chesson PL, 1985, Biological Control in Theory and Practice, The American Naturalist, Vol.125, Issue 3, pp.344-366

Nuzzo VA, 1997. Element stewardship abstract for *Cirsium arvense* (Canada thistle, creeping thistle, California thistle), [Online.] Available: http://tncweeds.ucdavis.edu/, Prepared for The Nature Conservancy, Arlington, VA, 30 pp.

Office of technology assessment, 1995, Biologically Based Technologies for Pest Control, OTA Publications, http://www.wws.princeton.edu/ota/ns20/year f.html.

Peschken DP, Wilkinson ATS, 1981, Biocontrol of Canada thistle (*Cirsium arvense*): releases and effectiveness of *Ceutorhynchus litura*(F.) (*Coleoptera: Curculionidae*) in Canada, Canadian Entomologist, Vol.113, pp.777-785

Peschken DP, 1984b, 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, Vol.116, pp.1377-1384

Pyšek P, Sádlo J, Mandák B, 2002, Catalogue of alien plants of the Czech Republic, Preslia, Vol.74, pp.97-186

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

Slavík B, Štěpánková J, 1998 - 2000, Květena Česke Republiky, Vol.7, p.1672, book

Torchin ME, Lafferty KD, Kuris AM, 2002, Parasites and marine invasions, Parasitology, Vol.124, pp.137-151

Turnbull AL, Chant DA, 1961, Practice and theory of biological control of insects in Canada, Canada Journal of Zoology, Vol.39, Issue 5, pp.697-753

Wandeler H, Bacher S, 2006, Insect-transmitted urediniospores of rust *Puccinia punctiformis* cause systemic infections in established *Cirsium arvense* plants, Phytopathology, Vol.96, Issue 8, pp.813-818

Wool D, 2002, Herbivore abundance is independent of weather? A 20-year study of galling aphid *Baizongia pistaciae* (*Homoptera: Aphiodoidea*), Population ecology, Vol. 44, Issue3, pp. 281-291

Zwolfer H, Eichhorn O, 1966, The host ranges of *Cassida* spp. (*Col. Crysomelidae*) attacking *Cynarea* (*Compositae*) in Europe, Zeitschrift für angewandte Entomologie, Vol. 58, pp. 384-397