

Interactions between the invasive weed *Heracleum mantegazzianum* and associated insects



Inauguraldissertation
der Philosophisch-naturwissenschaftlichen Fakultät
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Vorgelegt von
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Aus Dänemark

Leiter der Arbeit
Prof. Dr. W. Nentwig
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Der Dekan:
Prof. Dr. P. Messerli

To my mother

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General introduction

Heracleum mantegazzianum Somm. et Lev., giant hogweed (Apiaceae), is a tall perennial monocarpic plant that flowers in the third to the fifth year. *H. mantegazzianum* is native to the western Caucasus where it occurs in the upper forest belt, in meadows, clearings and forest margins (Mladenova, 1950). It was introduced to botanical gardens of the European countries in the 19th century (Pysek et al. 1998; de Waal *et al.* 1994) and has now naturalized near waterways, roads, fallow land and disturbed land all over Europe. *H. mantegazzianum* has firmly established itself in nitrophytic herbaceous perennial communities (Otte et al. 1998).

The centre of origin of the genus *Heracleum* is considered to be in the Caucasus region, where more than 12 *Heracleum* species and many subspecies have been described, while in Europe only 2 indigenous and 3 invasive species occur. Even specialists of this plant are confused by the numerous species descriptions, multiple introductions often via European botanical gardens, the large morphological variance within each *Heracleum* species and the hybridization between some species. Many species descriptions of the same species have resulted in several synonyms for *H. mantegazzianum*, and *Heracleum sosnowski* Manden considered a very close relative to *H. mantegazzianum*. The ongoing genetic analysis of this genera will perhaps solve some of these problems.

Plant dispersal and invasion: *H. mantegazzianum's* fruiting bodies (mericarps) are almost not found further away than 250 cm from the mother plant (passive autochory) (Otte et al. 1998). Long-distance hydrochoric dispersal is also likely to occur. On the contrary are anemochoric and zoochoric dispersal of little significance (Otte et al. 1998). Though not restricted from semi-natural vegetation, the species depends to a large extent on humans, regarding the large scale dispersal of seeds (Pysek et al. 1998). After a plant has been introduced, it will slowly establish in the new environment. After a lag phase, the spread of the species often becomes exponential (fig. 2).

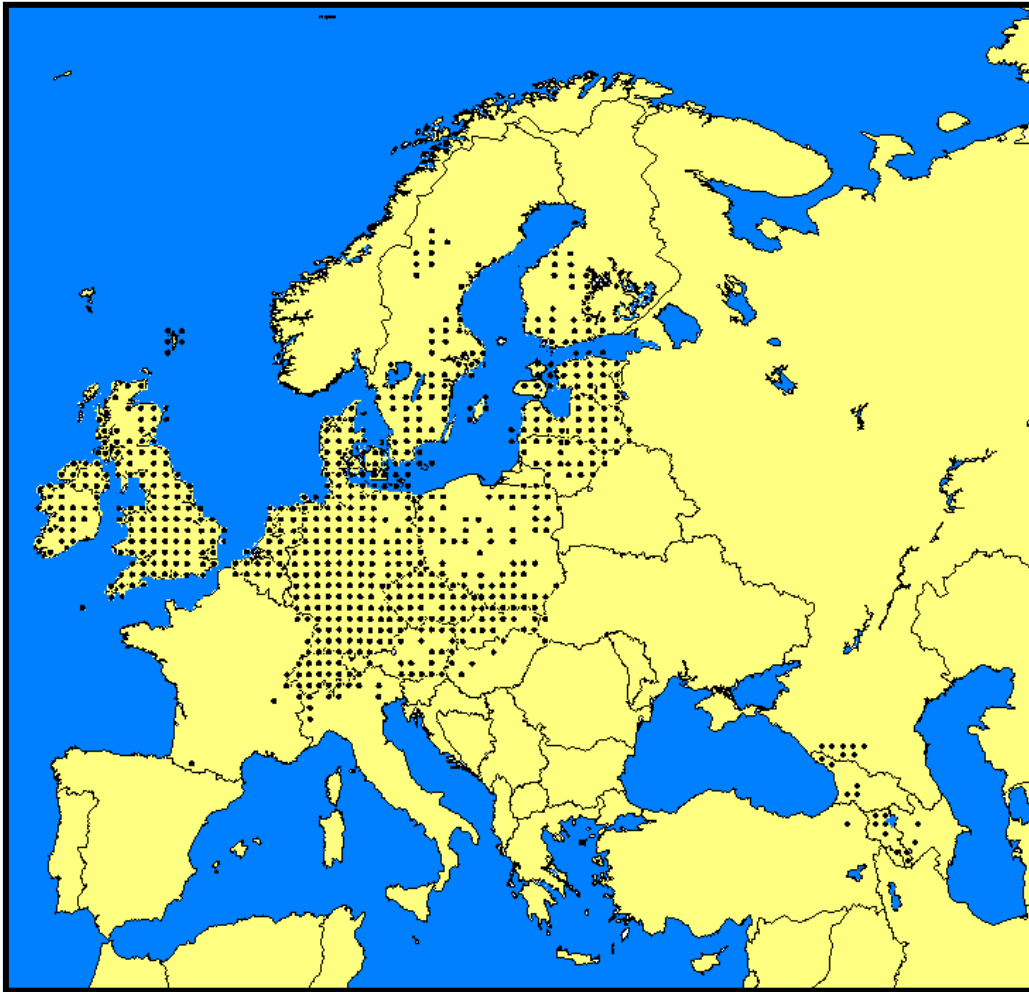


Figure 1: Distribution map of the neophytes *Heracleum mantegazzianum* and *H. sosnowskyi* in the invaded and native regions of Europe and the Caucasus (60 km x 60 km plots, Booy 2005, personal communication). Additionally *H. mantegazzianum* invaded large areas of the North American continent. The origin of *H. mantegazzianum* is in the north west of the Caucasus region and the closely related species *H. sosnowskyi* comes from the south east of the region, from the Transcaucasus.

Biological control: The replacement of native vegetation and the injuries to human skin caused by the secondary metabolites are the major reasons, why several countries in Europe recognize *H. mantegazzianum* as a serious threat and try to eradicate it (Pysek et al. 1998). To create a strategy that could prevent this weed from spreading further in Europe and to create a knowledge base, 6 European countries have united their effort, in the years 2002-2005, under the project name “Giant alien” (www.giant-alien.dk).

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The economic costs of treating and controlling giant hogweed in Germany exceeded 11 Mio Euro/year (Schepker and Kowarik 2001). When an invasive weed has reached the exponential phase it becomes difficult and often uneconomic to control by chemical and mechanical control methods. The *H. mantegazzianum* population in the Czech Republic seems to have reached this exponential growth about 50 years ago (figure 2) (Pysek and Prach 1993).

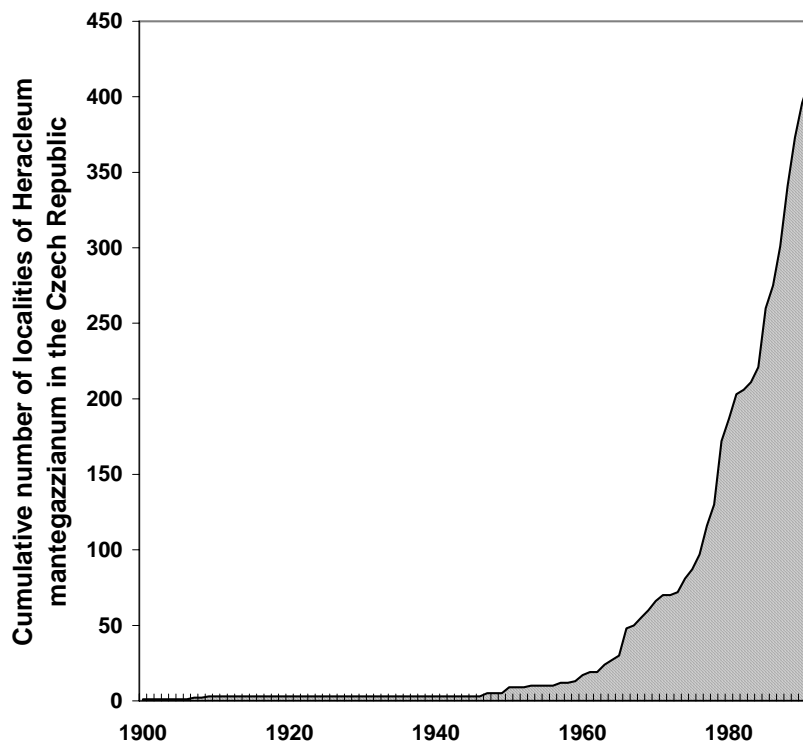


Figure 2. The number of reported localities of *Heracleum mantegazzianum* increased exponentially after 1950 in the Czech Republic (based on historical records)(Pysek and Prach 1993, Pysek 1994).

Many plants are limited in number by specialised phytophagous insects and when plants move into new regions they often become separated from these specialised herbivores. Consequently they increase radically in abundance and become invasive (van Driesche and Hoddle 2003).

Biological control can be implemented in four different ways: 1) conservation of natural enemies, 2) introduction of foreign species for permanent establishment (classical biocontrol), 3) temporary augmentation of enemies or 4) microbial pesticides. Augmentation of natural enemies with subsequent release in a particular area is predominantly applicable for high-value crops in greenhouses. Classical biological control has resulted in successful management of invasive plant species in several occasions (e.g. *Opuntia* sp. in Australia, *Euphorbia esula* L. in North America, *Salvinia molesta* Mitchell in the tropics). Until now, no attempts on classical biological control of plants had been made in Europe. Introductions of specialised herbivores, have been tried out against 133 species of invasive plants, primarily in Australia, America and Africa and of these 41 species (31 %) have been completely controlled (van Driesche and Hoddle 2003). To employ a phytophagous agent for classical biocontrol of weeds, there are several factors that must be known before release. The agent must be host-specific to avoid damage on non-target weeds and the relation of the agents to natural enemies should be known. Moreover is it an advantage, if the herbivore attacks the vulnerable organs or critical stages in the plants life cycle, such as *H. mantegazzianum*'s long lasting taproots or seedlings. It was shown by that severe attacks on seeds (between 70 - 99,9 %) are needed to prevent a plant from spreading (Parker 2000; Maron and Vila 2001). If for instance 90 % of the seed output from *H. mantegazzianum* in 2003, in Pregradnaja, Caucasus are destroyed (average seed number \pm SD = 2800 ± 1200 , n = 88) (Hansen 2005, appendix), there would still be 280 seeds left to disperse. The mortality (e.g. self-thinning effect, Otte and Franke 1998) of those seeds are not taken into account. Our estimates for the average seed-damage in the Caucasus is certainly much smaller than 90 %.

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Table 1: Neophytes in the German flora (Kowarik 2001)

	Plant species	
Flora of Germany	3062	100 %
Native species	2375	78 %
Non-native species	687	22 %
Achaeophytes (before 1500 A.D.)	275	9 %
Neophytes (after 1500 A.D.)	412	13 %

72 % of the German local authorities had problems with the non-native flora. 27 % of these were related to economic conflicts and 6 % were related to health problems (Kowarik 2001). About 15 out of the 31 controlled non-native species (non-agricultural) were specifically controlled by herbicides.

An inquiry among local authorities in Niedersachsen (northern Germany) showed that more than 2/3 of all reported invasions events are related to 3 taxa: *Prunus serotina* Ehrh. (Black cherry), *Heracleum mantegazzianum* and *Reynoutria* spp. (Japanese knotweed) (Schepker and Kowarik 2001).

Thirty-one non-native species were causing problems (non-agricultural) and in 222 out of 457 cases, mechanical and chemical controlling activities were carried out. During 12 years, 3.5 Mio Euro were spent to control 222 stands in Niedersachsen.

Table 2: Non-native species perceived as troublesome invaders in Niedersachsen, Germany (based on 457 invasion invents) and the extent and success of control activities during 12 years (Schepker and Kowarik 2001).

Species	No. of invasion events	Invasion events (%)	Attempted control (%)	Successful attempted control (%)
<i>Prunus serotina</i>	147	32	77	27
<i>Heracleum mantegazzianum</i>	82	18	63	21
<i>Reynoutria</i> spp.	81	18	30	18
<i>Impatiens glandulifera</i> Royle	29	6	10	100
<i>Elodea</i> spp.	19	4	42	0
Others	99	22	22	16
Total	457	100	49	23

H. mantegazzianum creates a serious problem, causing 18 % of all invasion events in Niedersachsen (table 2). The authorities in Niedersachsen are determined in stopping the weed and attempts to control giant hogweed in 63 % of the cases. The small success rate of 21 %, however, shows that an effective way of controlling giant hogweed is still urgently needed (table 2). The primary objective of my PhD thesis is to investigate the phytophagous insects and evaluate their potential as biological control agents. When doing such an evaluation it is important to investigate the interactions between the insects and plant. This is therefore also the main aim of this thesis.

Plant defence systems: Plant defences against herbivores can be divided into five categories: 1) physical defences such as thorns, trichomes, sclerophylly (tough leaves) etc. (Gullan and Cranston 2000, Valverde et al. 2001), 2) lowered levels of nutrients and water (water/nitrogen ratio)(Strong et al. 1984), 3) noxious phytochemicals (toxins or reducing digestibility)(Berenbaum and Zangerl 1994), 4) cryptic appearance or decreased conspicuousness (Karban and Baldwin 1997), 5) indirect defences by attracting parasitoids or predators (Pallini et al. 1998, Gullan and Cranston 2000) or attracting other beneficial organisms like ants, which repel herbivores (myrmecophytes, Jolivet 1996). Ants protect some plants from certain herbivores (Vasconcelos 1993, Halaj et al. 1997) but conversely protect trophobionts (homopterans) from predators or fungal attack (Hölldobler and Wilson 1990, Morales 2000).

Furanocoumarin defence compounds, found in plants from Apiaceae, are well-documented to reduce palatability and suitability of foliage to a wide array of both generalist and specialist herbivores (Berenbaum 1995). The toxicity of furanocoumarins is increased in the presence of ultraviolet light which catalyzes cross-linkage of the pyrimidine bases in the DNA strands (Berenbaum, 1978). The selective pressures of specialist herbivores adapted to the linear furanocoumarins have evolutionarily led to a new biosynthetic pathway among plants from Apiaceae, producing the more toxic angular furanocoumarins (e.g. angelicin) (Berenbaum 1981, Berenbaum and Feeny 1981).

Some plant defence systems are inducible by pathogens and/or herbivores (Gullan and Cranston 2000). If defence is costly (in energetic terms) and if insect damage is intermittent, plants would benefit from being able to turn on their defences only when insect feeding occurs. Induced resistance against herbivores has been described for over 100 plant species (Karban and Kuc 1999). The furanocoumarin content in *Pastinaca sativa* L. for example, (a close relative to *H. mantegazzianum*) has been shown to be inducible (Zangerl and Berenbaum 1998).

Plant-aphid-ant interactions: Ants are known to have a great impact on their habitats. Ants can protect plants from phytophagous insects, monkeys and even elephants (Janzen 1972), some cut leaves, pollinate flowers, disperse seeds, tend homopterans, grow fungus on leaf litter, create “ant-gardens”, build structures, aerate and fertilize soil, etc. (Hölldobler and Wilson 1990). Hundreds of ant-plant symbioses have been documented in the past 150 years and various plant structures have specially evolved in the coexistence with ants. Such plant organs are domatia, extrafloral nectaries, food bodies, elaiosomes eliciting myrmecochory etc. (Hölldobler and Wilson 1990). In the whole America ant-plants are found in 16 plant families and 35 genera (Benson 1985). Additionally ants frequently form intimate associations with homopterans. The other objective of this thesis is to investigate the interactions between giant hogweed and its associated insects, in particular the aphids and ants.

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PART I

**Three partner mutualistic relationship beneficial to all partners:
ant-made soil-shelters house aphids on giant hogweed**

Three partner mutualistic relationship beneficial to all partners: ant-made soil shelters house aphids on giant hogweed

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Abstracts

The aim of this field study was to investigate, with a multivariate factorial design, the interactions between giant hogweed (*Heracleum mantegazzianum*), myrmecophilic aphids, ants, and non-myrmecophilic aphids. The ant *Lasius niger* improves the leaf envelope capacity (domatium) of giant hogweed by building above ground soil shelters to lodge colonies of the obligate myrmecophilic aphid *Anuraphis subterranea*. Controlled by the domatium size, the aphid population cannot seriously harm the plant. We found a positive correlation between the relative plant growth, the ant activity, and the number of myrmecophilic aphids inside the domatium. On the other hand, two non-myrmecophilic aphid species on the leaves, *Paramyzus heraclei* and *Cavariella theobaldi*, reduce the growth of giant hogweed in the native habitats. The ants are then again limiting this damage because we found a negative correlation between ant activity and aphid numbers on the leaves. Only few field experiments have described systems where three partners benefit from the mutualistic relationship simultaneously. In contrast to the classic experiment of Messina (1981) the here reported three partner mutualistic relationship appears to be more specifically adapted and it probably provides a more stable benefit to the plant. Since domatia have so far only been described from the tropical or subtropical regions this is the first report for a domatium from the temperate zone. It is, moreover, the first experimental result supporting

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the hypothesis that the abundant tropical secondary domatia initially have evolved via a three-species symbiotic relation between plant, aphid and ant.

Many tropical plant species have scale insects (Coccoidae) and treehoppers (Membracidae) which attract ants with their honeydew. Some ants enter a trophobiosis with homopterans which provide the ants with food indirectly via the plant (1-2) and honeydew is known to provide a large percentage of the energy budget for ant species (3-5). Ants protect plants from certain herbivores (6-7), and homopterans from predator or fungal attacks (1, 8-9). Ant tended aphids and soft scales remove plant sap and may damage plant tissues or inject toxins and attract pathogens (9-10). On the other hand, plants bearing ant Homoptera associations may experience reduced herbivore damage (9), as plants with extrafloral nectaries do (11-12). The ant *Lasius niger* L. has a strong positive effect on the growth and speed of maturing of the aphid *Aphis fabae*, it quickly repels adult coccinellids from aphid aggregations, and carries off small coccinellid larvae (13).

Ants obligately occurring inside living plants have evolved in five out of 12 ant subfamilies in approximately 30 genera (14). Some ant families are perhaps predisposed to evolve symbiotic associations with homopterans or plants (14). The hollow space or crevice inside the plant lodging beneficial insects is called a domatium (15-18). Two types of ant domatia are distinguished: In the case of a primary domatium ants invade a weak plant structure (e.g. hollow stems) and instantly provide protection. A secondary ant domatium is an inflated or modified structure that has coevolved over a longer period, specifically to specifically house certain ant species (16).

Benson (19) proposed that the homopterans are zoological devices used by the plant to maintain the ants near the plant, even if he was not able to see how the domatia, which were too small to shelter aphids or ants, could evolve in the beginning. For such a symbiotic balance to be stable, there must also have been a selection for a system where the homopteran populations cannot grow unlimitedly and kill the plant, and a system where the plant does not defend itself so vigorously that it starves the ant colony (20). Domatia may also provide protection to the coccids from inclement weather conditions, from predators and parasitoids, and they may reduce the incidence of diseases (10, 17, 21). Many tropical ants protect their

colonies and their honeydew sources by building plant-fibre shelters, thus extending their caring effort into the rainy periods (19, 22).

Plants with domatia (myrmecophytes) are widespread in tropical and subtropical plant families in Africa, America, Australia, and Asia. Domatia have never been found on plants from Europe (1-2, 14, 16, 23) probably because it is impossible to reside in a plant all year around under the climate regime of the temperate zone.

Giant hogweed *Heracleum mantegazzianum* Sommier and Levier (Apiaceae) is native to the Western Caucasus where it occurs in the upper forest belt, mainly in meadows, clearings, and forest margins (24). *H. mantegazzianum* is a tall and fast growing monocarpic perennial plant with a rapid leaf turnover (25-26). The good competitive ability and high seed production makes it an aggressive invasive species, especially in habitats where the land use is changing. In the 20th century it invaded and naturalized near waterways, roads, on fallows, and in disturbed landscapes in Europe and Northern America (24).

Among the diverse insect fauna associated with *H. mantegazzianum* are more than 13 aphid species, and eight of these are specialised on Apiaceae (27). The four most common aphids divide their feeding niches on *H. mantegazzianum*: *A. subterranea* Walker and *Dysaphis lauberti* Börner only feed in the leaf envelope (domatium). The non-myrmecophilic aphids *Cavariella theobaldi* Gillette and Bragg and *Paramyzus heraclei* Börner feed on the leaves or umbels sometimes in large densities. *A. subterranea* has specialized in this niche since the apterous viviparous have a very long rostrum (0.7 x body length, 28) which allows penetration of the thick epidermis of the stem base of *H. mantegazzianum*. The leaf-feeding *C. theobaldi* and *P. heraclei* have a rostrum half this size. Ants only build soil shelters on top of the domatium when aphids have entered the envelope. *A. subterranea* individuals are normally located very quickly by the ants because of the relatively high ant densities on the ground.

One hypothesis for the function of the leaf envelopes of *H. mantegazzianum* is that it protects the aphid *A. subterranea* against parasitoids or predators. Alternative hypotheses could be: a) protection of the vulnerable petiole shoots in their early developmental stages, b) mechanical structure that keeps the leaves in the right position, c) protecting aphids and ants against strong humidity and temperature changes, thus reducing the risk of desiccation or

extreme heat, d) UV-light protection. The plant sap contains furanocoumarins (29) which in combination with UV light are toxic. Ants and aphids could perform better when they are protected against sunlight. These hypotheses are not mutually exclusive and we did not test the alternative hypotheses in this study. Instead we are focusing on the net outcomes of the relations between plant growth, ant activity, and populations of non-myrmecophilic and myrmecophilic aphids.

The following four hypotheses were tested in our field experiment with a multifactorial design: 1) Each of the aphid species has a negative impact (ants are neutral) on the plant growth. Manual aphid removal and removal with insecticide will therefore lead to increased plant growth. 2) Ants have a positive impact on the plant and are positively correlated with them. Repelling the ants with insecticide in the domatia will therefore reduce plant growth. 3) Nutrients benefit both aphids and plants. When soil nutrients enter the plant it will lead to increased plant growth but simultaneously stimulate the growth of the herbivores. The negative effect of more aphids feeding on plant growth will however be smaller than the positive effect of more nutrients for plant growth. Therefore by adding fertilizer both plant growth and growth of aphids will increase. 4) Ant-made soil shelters are beneficial for the aphids, but have no impact on the plant growth. Artificial clay shelters have a similar effect on plant growth and aphid numbers as ant-made soil shelters do.

We found a three partner mutualism between plant, ants, and the myrmecophilic aphids where each of the partners profit from the others. Our setup allows us also to estimate the different impact of the two aphid groups (in domatia and on leaves) on plant growth, in order to evaluate their suitability as biological control agents of the invasive weed *H. mantegazzianum*.

Materials and Methods

The main experiment was set up 2003 in Pregradnaja at the northern slope of Caucasus in Russia (N 43° 54' 26'', E 041° 17' 03''). Additional observations and investigations concerning the plant-aphid-ant interactions were made 2002 and 2004 in the Georgian and Russian Caucasus, in Switzerland, Czech Republic, Netherlands, Belgium and Latvia. The main study locality is a 2 ha clearing partly surrounded by forest, the Teplaya River and a

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road. The area is a partly abandoned agricultural field where plants such as *Petasites hybridus* L., *Chaerophyllum aureum* L., *Alcea rugosa* Alef., and *Dactylis glomerata* L. were abundant. We randomly selected *H. mantegazzianum* plants with soil shelters (hence also with aphids and ants). For each plant, the individuals of *C. theobaldi* and *P. heraclei* feeding underneath three leaves and *A. subterranea* feeding in the leaf envelope were counted. The average increase in *C. theobaldi* and *P. heraclei* feeding on the leaf underside of the smallest, biggest and the medium sized leave respectively, were calculated. By assuming that the large numbers of aphids in the umbel and flower buds are closely correlated with aphids on the leaves, we could avoid the time consuming and difficult task of counting aphids in the umbel to get the total number of *C. theobaldi* and *P. heraclei* aphids. Individuals of *A. subterranea* in the leaf envelopes were counted and the ant activity was measured as the number of ants running on the ground within a radius of 10 cm from the stem base, during one minute. All data were collected daily between 9 AM and 5 PM.

To be able to compare the relative growth of the plants, the following plant variables were measured at the end of June and one month later, at the end of the experiment: Plant height above soil surface, number of leaves, length and width of the largest leaf, diameter of the terminal umbel, number and weight of the seeds on the terminal umbel at the end of the experiment. The number of seeds was estimated for each plant by counting the seeds on 9 umbellets in the primary umbel (3 umbellets from the periphery, 3 from the centre and 3 from in between). The total number of umbellets was counted and then the approximate total number of seeds on the terminal umbel could be calculated. After the growth variables were z-standardized, the mean of these standardized plant variables was calculated to get a single value describing the plant growth. By combining all seven plant variables into one, it is possible to give a more precise estimate of the total increase in plant size, even when the plant parts grow differently. To make interpretation easier, the value of 1 was added to the mean normalized values for relative plant growth in Fig. 2a and 3a.

The experiment had a 3 x 4 factorial design, where 1) insecticide added, 2) manual removal of aphids, and 3) control were combined equally with the following factors: a) soil shelter removal, b) constructing artificial shelter, c) adding fertilizer, d) control. Treatment description: 1) A non-systemic insecticide (2 ml 0.0055 % cypermethrin) was applied to the

soil-shelter and leaf envelope at the stem base. 2) Aphids in the leaf envelope were carefully removed by means of a forceps. These factors were combined with the following factors: a) Soil shelters were removed manually with a spoon. b) Clay shelters were formed artificially to cover the envelopes, with small openings to allow ants to keep tending the aphids. c) 20 g fertilizer containing 15 % N, 5 % P₂O₅, 18 % K₂O, 1.4 % Mg, 0.1 % B, was added in a water solution to the treated plants. All 3 x 4 combinations were applied randomly to the 96 selected three year old plants, because only older plants have an envelope open enough for the ants and aphids to enter. Each treatment factor combination had 8 replicates per cell. Plant growth, aphid numbers in domatia, aphid numbers on leaves, final soil shelter size and ant activity were included in a model III MANOVA as dependent variables (using SPSS 12.0). Post hoc Tukey tests were performed after the MANOVA comparisons. A 10 block MANOVA design was conducted to incorporate the effect of having slightly variable soil and microclimatic conditions at the locality. Because of skewed normal distributions, the numbers of aphids on leaves and the ant activity on the ground were logarithmically transformed. In multiple Spearman's rho correlations the critical p-values were adjusted after Bonferroni (31).

Results

H. mantegazzianum possesses a domatium made up by the petiole envelopes at the stem base, regularly sheltering the exclusively myrmecophilic aphid *A. subterranea* and a number of tending ants. These envelopes are curved inwards to form a cavity partly covered from above and coated by the ants with soil particles. Exactly in this part of the envelope *A. subterranea* is normally found (Fig. 1). The domatia start appearing on the *H. mantegazzianum* plants when they are approximately one year old. In the flower setting stage the envelopes enclose volumes up to 50 cm³. In July the ant-made soil shelters had a mean surface area of 18 ± 23 cm². During our investigations *L. niger* were tending 57 % of the plants with *A. subterranea* aphids and *M. rubra* or *M. ruginodis* were tending 39 % of the plants. In 4 % of the plants we observed more than one ant species near the soil shelter.

The insecticidal and manual removal of aphids significantly reduced the numbers of *A. subterranea* as expected (Fig. 2d), and both of these treatments simultaneously reduced the plant growth (Fig. 2a, 2b) (MANOVA and Tukey test). When fertilizer was added, the plants

grew significantly more (Fig. 3a). By removing the shelter the number of aphids (Fig. 3b) as well as the plant growth increased (Fig. 3a). But adding fertilizer or constructing artificial clay shelters over the envelope did not affect the number of aphids inside a domatium significantly (Fig. 3b). None of the performed treatments had a significant impact on the logarithmic transformed ant activity when performing the MANOVA.

The numbers of *A. subterranea* had a positive impact on the plant growth and showed a significant correlation between growth and aphid numbers (Table 1). This can be explained by the numbers of *A. subterranea* which are correlated significantly with the ant activity. In contrast, there is a negative correlation between the number of aphids on the leaves (*P. heraclei* and *C. theobaldi*), the increase in plant height (cm), and the ant activity (Table 1). An average of 82 ± 79 aphids ($\bar{X} \pm SD$) were feeding on the leaves. The MANOVA with the following post hoc Tukey test, however, showed that no treatments had a significant impact on the number of aphids on the leaves (Fig. 2f, 3c). The constructions of the artificial shelters did not have impact on this system (Fig. 3a, 3b, 3c).

Discussion

Plant-aphid-ant interactions. Our results (Table 1) support the second hypothesis which stated that ants are positively correlated with plant growth because ants protect the plant. An alternative explanation to the positive correlation between ant activity and plant growth is that ants supply nutrients. The soil shelter removal and applications of insecticide could somehow influence the bioturbation made by the ants in this area. Ants are known to turn and aerate the soil, they add nutrients in form of excrements (16, 19) and they hold temperature and humidity at moderate levels. Larger ant nests are often surrounded by a species-rich vegetation but only a few quantitative studies have been conducted to estimate the effect of this nutrient enrichment (1). In our experiment the ants did not build real nests but much smaller outposts with only small amounts of transported soil. The amount of excreta produced by so few ants is also low. Since removing soil shelters has a high impact on the plant growth (Fig. 3a), other so far unknown interactions could exist beside the nutrient explanation. Alternatively the correlation between ant activity and plant growth could be a

result of the combined effect of both factors; ant related fecundity/mortality for the herbivores and increased bioturbation.

Myrmecophilic aphids exert two opposed forces on the plant growth: one detrimental effect by feeding on plant sap and one beneficial effect by attracting ants. The significant positive correlation between the numbers of *A. subterranea* and the increase in plant height (Table 1) indicates that the positive effect of ants is stronger than the negative effect by aphids. The non-myrmecophilic aphids have a negative impact on plant growth and consequently we can accept our first hypothesis only for non-myrmecophilic aphids.

Ants frequently build protective covers over aphid and coccid aggregations (10, 21). In our system an important valuable effect is that the ant made soil shelter prevents the domatium from getting flooded during rain. Artificial domatia also increased the numbers of predaceous bugs to the benefit of the plant (17, 31), whereas herbivores such as aphids, spider mites and whiteflies decreased in numbers. Finally, the ant-made and the artificial shelter were shown to have similar effects on aphid numbers and plant growth (figure 3a, 3b) and concerning this point the fourth hypothesis is accepted. However, removing the soil shelters led against our expectations to an increased plant and aphid growth inside the domatia (figure 3a, 3b).

The negative correlation between plant growth and the increase in number of aphids on the leaves (*P. heraclei* and *C. theobaldi*) indicates that they damage the plant (Table 1). The average of 82 aphids on the leaves (which we analyzed carefully) can probably not have the observed impact alone, but this number is closely correlated with the much higher number of aphids in the umbels (which we did not analyze in detail). Both groups together cause the observed impact. When searching for a potential control agent for a biological control of the invasive populations of giant hogweed in Europe, such high aphid abundances generate the question as to whether these aphids could not control giant hogweed after the ants have been removed. Besides the problem that a selective ant removal would be difficult, removing ants would first result in a reduction of *A. subterranea* aphids beneficial to plant growth, and secondly it would lead to an increase of *P. heraclei* and *C. theobaldi* aphids which have a negative impact on plant growth. The resulting impact could be severe enough to control the plant if it occurred as early in the season as possible. This could be achieved by an early

augmentative release of *P. heraclei* and/or *C. theobaldi*). However, these aphids are oligophagous, i.e. not sufficiently species-specific, and side-effects on non-target host plants (e.g. *Pastinaca sativa*) would occur. So we conclude that the here presented aphid association is unlikely to control giant hogweed.

A few aphids still existed after insecticide application or manual removal (Fig. 2d). Obviously some survived the treatment or some migration to the treated plants occurred. In spring or early summer alate individuals of *A. subterranea* migrate from the primary (*Pyrus communis* L.) to the secondary host plant (giant hogweed). The new population built up consists chiefly of apterans. In July, we found less than 0.2 % alates, and these had crippled wings (probably chewed on by the ants to prevent migration, 32). We therefore assume that apterous aphids did not migrate in large numbers to other plants during the experimental period. Hence the correlation of aphid numbers with plant growth is primarily a result of the treatments; and not of aphid migration towards faster growing plants. Aphids probably would prefer fast growing plants, as their sap contains higher amounts of nitrogen and sugars. This influences quality and quantity of the rewards produced by the herbivore and hence the degree of protection that the ants provide to them in return (33-34). However adding fertilizer to the plants did not increase the aphid population significantly in this experiment (Fig. 3b, 3c). We can probably exclude an insufficient nutrient uptake by the plants because plant growth increased (Fig. 3a) and so we assume that the applied nutrients do not limit aphid population growth. Hence hypothesis three is accepted concerning the plant growth but rejected for both types of aphids.

Mutualism. If the net effect of an interaction between two partners is positive, i.e. increased fitness for both partners, this interaction is called mutualism. For example, the presence of ants has been shown to improve feeding and growth rate, survivorship, and fecundity of homopteran colonies (7) and the ants also profit from the homopterans they tend (1). It would seem impossible for an aphid colony to live inside a domatium without tending ants because pathogens spread when honeydew is accumulating. Ants remove the honeydew and produce an antibiotic substance from their metapleural glands (10).

It is more difficult to accept that a plant is also benefiting from a tritrophic interaction which involves herbivores. So far this has only been really demonstrated in a few cases, (34, 11). Our giant hogweed example is similar to the example of Messina (34) where goldenrod (*Solidago* sp., Asteraceae) is protected as a consequence of the mutualism between ants and membracids. Unlike plants in typical ant-plant mutualisms, goldenrod possesses no visible adaptations to attract membracids and accompanying ants. *H. mantegazzianum* is more involved in the mutualism because it offers protected feeding sites to the specialised aphids *A. subterranea*, and also shelter to the ants. In many ant-homopteran-plant interactions the ant/homopteran mutualism exerts an overall negative effect on plant growth and seed setting (36-37). Other examples of mutualistic interactions between three partners have been described where plant growth is either negatively influenced by a third species or two partners get benefit even when the third species is not present (see recent review 35). In our *H. mantegazzianum* system the plant and the aphids are only mutualistic in the context of their association with the ants and all three partners have to be present for a positive net effect for all (Fig. 4). To the best of our knowledge, this is one of the rare cases of a tritrophic mutualistic interaction with benefits for all three partners at the same time.

Coevolution? It has been postulated that leaf-pouch domatia of ant-plants have evolved from acarodomatia (domatia that shelter mites) (14, 31, 38). However the domatia on *H. mantegazzianum* support the hypothesis by Benson (19) that leaf-pouch domatia may have evolved from small depressions of leaf surfaces sheltering ant-tended homopterans. But what is preventing *A. subterranea* colonies from over-exploiting the plant and maintaining the relationship as a mutualism? *A. subterranea* only seldom appear outside the domatia or ant-produced soil shelters near the stem basis. This may indicate that outside these areas they do not do well. Trichomes are only situated outside of the domatium (figure 1) and they are probably inhibiting aphids from sucking. Domatia are the only aboveground plant organ lacking trichomes and this indicates the intimacy of the mutualistic relationship. Thus the number of aphids in a domatium is regulated by the size of the domatium, i.e. by the plant itself.

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A. subterranea aphids are the most critical and most specialised partners in this three partner mutualism and they are specifically adapted to the leaf envelopes of a few species in the genera *Heracleum* and *Pastinaca* (Apiaceae). Like the dark red envelopes on *H. mantegazzianum*, *A. subterranea* is also reddish and consequently more cryptic to visually hunting predators. In contrast *L. niger* and *M. rubra* are among the most widespread and non-specialised ant species and they tend numerous aphid species in various habitats. Giant hogweed often grows in habitats where at least one of the two ant species normally lives and they are therefore not limiting the mutualism. *L. niger* is also a good partner for the plant because it is a relatively aggressive ant which provides good protection against herbivores (14).

It has been argued that the evolution of domatia can easily be understood via primary domatia where ants invade cavities of weak plants and instantly provide protection to the plant. The evolution of secondary domatia is different and needs more time to evolve (19). The leaf envelopes of *H. mantegazzianum* are not simple natural cavities (primary domatia) but rather the product of joint adaptations and co-evolution. All three partners in this system are regularly found together and all partners benefit simultaneously from each other. *A. subterranea* is the most specialised partner (e.g. by colour and specialized mouth parts) and the leaf envelope of *H. mantegazzianum* looks like an ideal aphid and ant attracting organ. Because it is small, it is not possible to house too many aphids which could damage the plant seriously and it is hairless and situated close to the ground where the probability of discovery by ants is high. The ants even spend additional energy to improve the leaf-envelope by constructing soil-shelters around the aphid colonies. This system also explains how some of the tropical ant-plants with secondary domatia could have evolved without e.g. extra floral nectaries, fruit bodies or excavated plant cavities as assumed by Jolivet (16).

If a mutual partnership is beneficial for a plant and a sheltering domatium provides higher fitness for plants, aphids and ants, probably there is a natural selection towards inflated envelopes which protect even better. One could also expect a further enlargement of the envelope into an even more distinct organ leading to a more obligate mutualistic relationship. If attracting the aphids is more costly than only attracting the ants, we can predict that during the further evolution the plant will try to attract ants without attracting the aphids.

This scenario is often encountered in tropical ant plants (16). The leaf envelope of *H. mantegazzianum* is folded inwards in a similar manner, but to a much lesser extent, than the leaf-pouch domatium observed at the base of *Delpydra macrophylla* Pierre (Sapotaceae) in southern Cameroon housing the timid ant *Technomyrmex* (14). This is an example of one of the later steps in the evolution of a secondary domatium.

A too specialised mutualistic relationship is perhaps not ideal for the opportunistic nature of *H. mantegazzianum* which is usually invading disturbed areas and growing in a temperate climate where a mutualistic relation comes to an end every autumn when the aphids migrate to their primary host and all above ground plant parts die. This yearly break down has been considered as the main reason as to why domatia have not distributed into the temperate regions (16). To the best of our knowledge the *H. mantegazzianum* case is the first description of a secondary domatium structure from the temperate zone. We also interpret this three-species mutualism in a coevolutionary way even if it has been questioned if multi-trophic/multi-species mutualisms can undergo coevolution at all (2).

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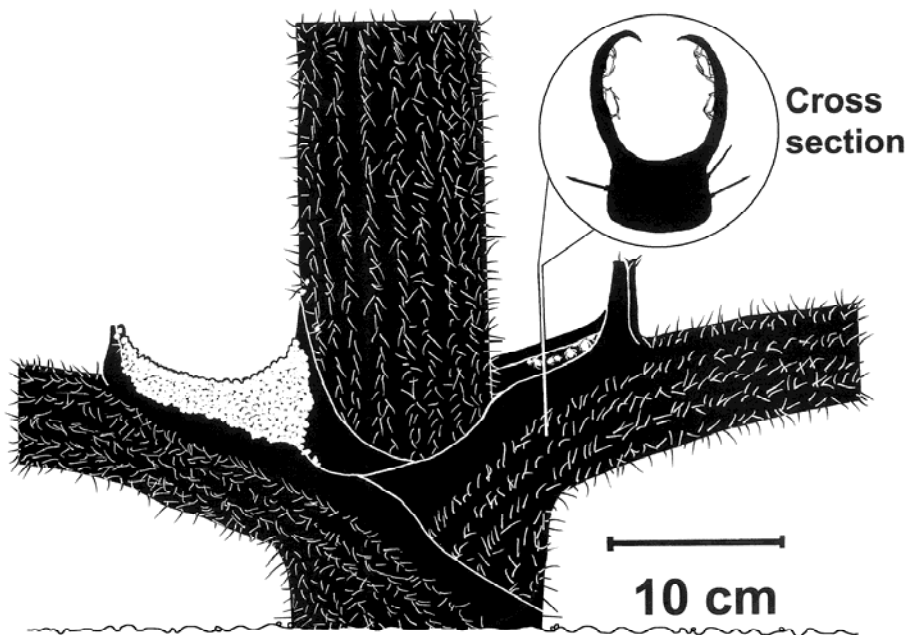
Part I

Table 1: Spearman's rho correlation coefficient for 4 variables (2-tailed significance, $n = 96$). α threshold values adjusted for multiple comparisons after Bonferroni: highly significant ** $p < 0.0017$, significant * $p < 0.0083$, marginal significant ^{ms} $p < 0.017$.

	Ant activity (individuals/min)	<i>A. subterranea</i> (numbers in domatia)	Aphids on leaves (numbers)
Increase in plant height (cm)	0.15 (0.014) ^{ms}	0.29 (0.0032) *	- 0.33 (0.0007) **
Ant activity (individuals/min)		0.28 (0.0041) *	- 0.29 (0.0030) *
<i>A. subterranea</i> (numbers in domatia)			- 0.10 (0.36)

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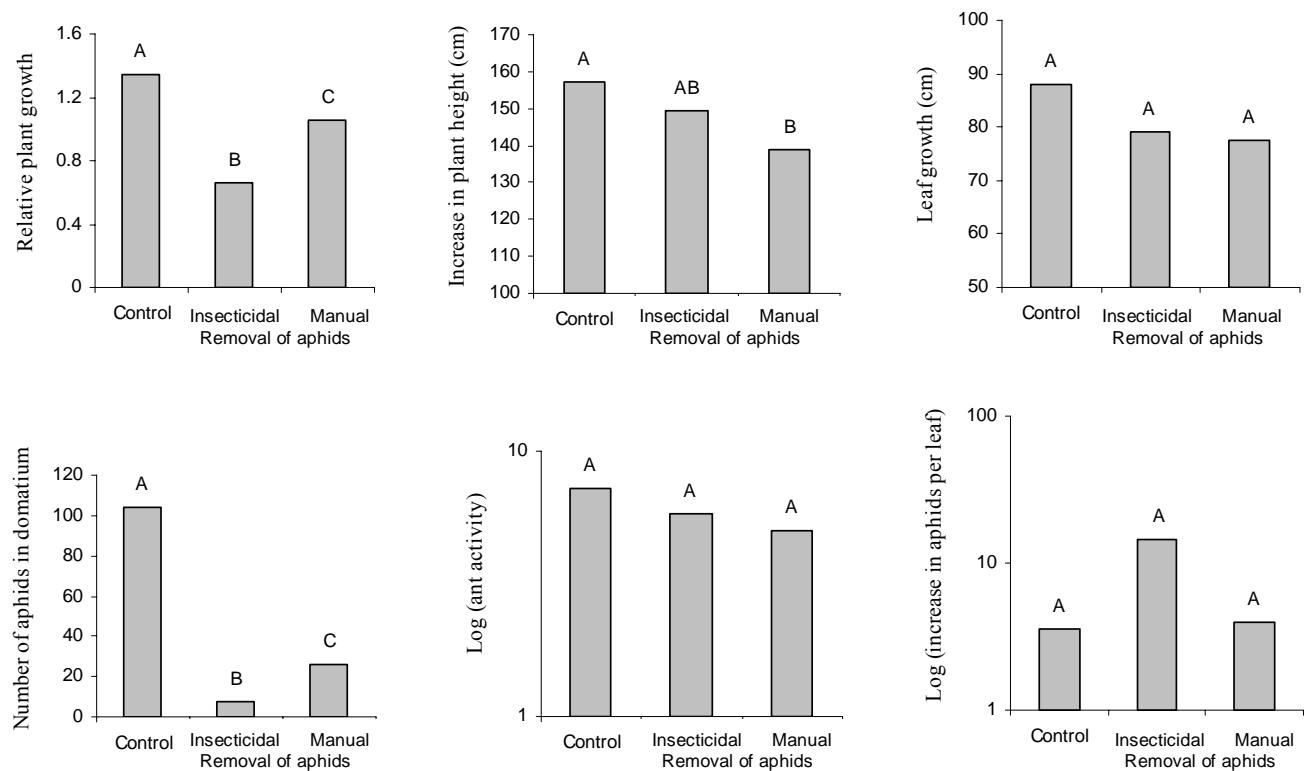
Fig. 1: Domatium (leaf envelope) is a hollow cavity at the stem base of 2-3 year old *Heracleum mantegazzianum* plants, sheltering colonies of aphids (*Anuraphis subterranea*) and ants (*Lasius niger* or *Myrmica spp.*). The domatium is curved inwards (see cross section) and ants construct soil shelters on top of the domatium (leaf envelope left side) when aphids are inside.



Part I

Part I

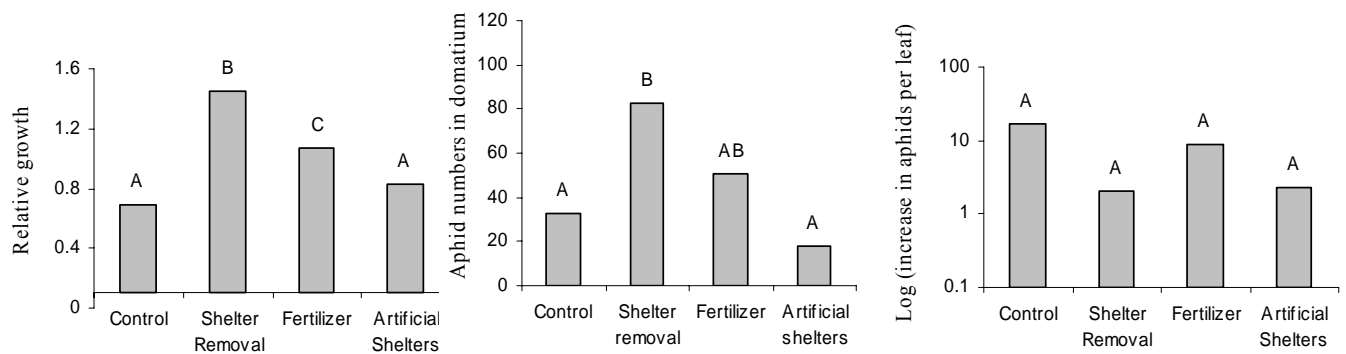
Fig. 2. Impact of insecticidal and manual removal of aphids feeding on leaf envelopes of *H. mantegazzianum* on: (a) relative plant growth, (b) increase in plant height (cm), (c) leaf growth (cm), (d) final number of aphids in domatia, (e) ant activity (min^{-1}), (f) increase in aphid numbers per leaf. Different capital letters indicate significance after a MANOVA and a post hoc Tukey test, $\alpha = 0.05$.



Part I

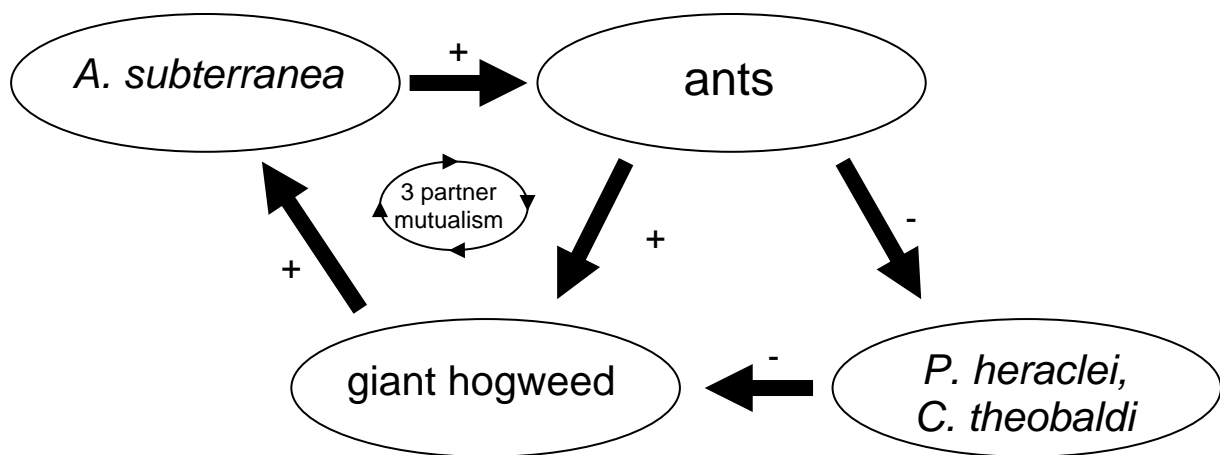
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Fig. 3. Impact of soil shelter removal, application of NPK-fertilizer and building artificial clay shelters on: (a) increase in plant height (cm). (b) final number of aphids in domatia. (c) log (increase in aphid numbers per leaf). Different capital letters indicate significance after a MANOVA and a post hoc Tukey test, $\alpha = 0.05$.



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Fig. 4. Net growth interactions between four different partners: The ants (*Lasius niger* and *Myrmica* spp.), the myrmecophilic aphid (*Anuraphis subterranea*) and the non-myrmecophilic aphids (*Paramyzus heraclei* and *Cavariella theobaldi*) and giant hogweed (*Heracleum mantegazzianum*).



PART II

Phytophagous insect fauna of the Giant Hogweed *Heracleum mantegazzianum* in invaded areas of Europe and in its native area of the Caucasus

Phytophagous insect fauna of the Giant Hogweed *Heracleum mantegazzianum* in invaded areas of Europe and in its native area of the Caucasus

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Abstract

Giant hogweed *Heracleum mantegazzianum* (Apiaceae) was introduced from the Caucasus into Western Europe more than 150 years ago and has meanwhile invaded many countries. To collect the phytophagous insects from different plant organs in the native range of giant hogweed (Caucasus) and to compare them with the herbivores found in the invaded parts of Europe, we visited about 27 localities in nine countries once or several times during two seasons. In addition, literature records for herbivores were analysed for a total of 16 *Heracleum* species. On the basis of a list of 264 herbivorous insects feeding on these *Heracleum* species, we describe here the herbivore communities, locate vacant niches and point out suitable biological control agents as a basis for the future control of *H. mantegazzianum*.

Similar studies of herbivores on invasive weeds showed that a larger proportion of specialist herbivores were found in native habitats compared to the invaded areas, and hence support both the enemy release hypothesis (ERH) and the evolution of increased competitive ability hypothesis (EICA). Only small differences in species composition were observed between Europe and the Caucasus, probably due to the proximity of the two regions and due to the lack of geographic barriers dividing them. Fewer herbivore species were found on the stem and roots, and more on the leaves, compared to the relative size of these niches. Most herbivores are generalists (polyphagous), some were found to be oligophagous, a few had only *Heracleum* species as host plants (monophagous) and none are known to feed exclusively on *H. mantegazzianum*. The oligophagous herbivores are restricted to a few taxonomic groups, especially to the Hemiptera which were particularly abundant on this weed.

Key words: Apiaceae, biological control, enemy release hypothesis ERH, evolution of increased competitive ability EICA, generalist, herbivores, invasive weed, monophagous, oligophagous, polyphagous, specialist.

1. Introduction

1.1. Giant hogweed

Heracleum mantegazzianum Sommier and Levier (Apiaceae) is native to the western Caucasus where it occurs in the upper forest belt, mainly in meadows, clearings, and forest margins (Mladenova, 1950). The plant was introduced to botanical gardens of some European countries in the 19th century (Pysek, 1994), and has now naturalized along waterways and roads and on fallow and disturbed land all over Europe. The good competitive ability and high seed production makes it an aggressive invasive species, especially where the land use is changing. It is a typical representative of the competitive/ruderal strategy type (Otte and Franke, 1998). The main reasons to stop this weed from spreading further in Europe are that the plants cause severe damage to the human skin when the furanocoumarins from the plant

sap react with DNA. Additionally invasive species affect the structure and function of an ecosystem and can also reduce the biodiversity of communities and landscapes (Pysek and

Pysek 1995). Furthermore erosion can increase when giant hogweed spreads along waterways and large areas of fallow land will be lost for other purposes.

The area of origin hypothesis assumes that in the evolutionary centre of a given species the number of congeneric relatives and the number of specialized herbivores is highest (Nentwig et al., 2004). The centre of origin of the genus *Heracleum* is considered to be in the Caucasus region, where more than 12 *Heracleum* species and many subspecies have been described, while in Europe only 2 indigenous and 2 invasive species occur.

H. mantegazzianum has at least two defence systems against herbivores. The first is the chemical defence system, made up of furanocoumarins (Berenbaum and Feeny, 1981) which are found in all plant organs at high concentrations (Knudsen 1983). This defence acts against internal and external feeders. Secondly, the plants have rows of hairs of varying lengths (0.5 μm – 7 mm) on leaf edges, leaf veins, and on the stem. This defence mechanism acts against external feeders. Both systems mainly deter generalist herbivores from feeding and determine the numbers of herbivore species on the plants (Lawton, 1976).

1.2. Weed invasion hypotheses

Blossey and Nötzold (1995) attributed the increased competitive ability of non-indigenous plant species to the absence of their specialized natural enemies. The enemy release hypothesis (ERH, Keane and Crawley, 2002) assumes that if no effective specialised antagonists are following an invasive weed, the plant experiences less regulation by specialized herbivores than the opponents, thus getting a competitive advantage. The slightly different and not mutually exclusive evolution of increased competitive ability (EICA) hypothesis, as modified by Müller-Schärer et al. (2004), claims that plants invest significant resources into herbivore defences in their indigenous environment. The weed's increased fitness, in an invaded region, makes the population grow and it may become invasive on an evolutionary scale. Both hypotheses are based on the assumption that there is a lower number of specialized herbivore species and/or lower individual abundance in the invaded area (Memmott et al., 2000). In the present paper we want to test if there is a larger proportion of specialists in the native region, and we want to confirm if this basic species assumption is true. These assumptions have rarely been tested (Mitchell and Power 2003). Wolfe (2002)

was probably the first to test if naturalized plants experience greater attack by enemies in the native region. Wolfe confirmed a higher level of attack by herbivores and pathogens on white champion (*Silene latifolia*, Caryophyllaceae) in its native range. Mitchell and Power (2003) demonstrated that the invasiveness of weeds is correlated with the release from pathogens. Besides providing a list of associated herbivores, the aim of this paper is also to test the hypothesis that proportionally more species of herbivore specialists are found in the native habitats. The final aim is to investigate if vacant niches on *H. mantegazzianum* do occur in Europe which could then be target niches for a biological control project.

2. Materials and methods

2.1. Study areas

The field surveys were carried out in Belgium, Czech Republic, Denmark, Germany, Holland, Latvia, Switzerland, and in the Caucasian areas of Georgia and Russia. In the period from early May to September 2002 until May to mid of August 2003, data from 37 different locations were acquired, 21 localities in Europe and 16 in the Caucasus up to 2050 m asl. An approximately equal amount of time was used to collect insects in the Caucasus compared with Europe. The fresh biomass of the plant organs (umbel, leaves, stem, root) was determined for 78 three year old plants and 64 two year old plants. Plants were chosen equally from two localities in the Russian Caucasus and four localities in Mariánské Lázně Czech Republic in the period from mid July until mid August 2003.

2.2. Insect collection and data analysis

Ten plants were investigated per location and many of these locations were visited more than once during the two seasons. The leaf surface, stem, and umbel were searched with aspirator and forceps. After this, the stem and petioles were dissected to uncover internal stem feeders. Finally, the root was dug up and carefully sliced to find external and internal root borers. Larvae were reared to adult stage in climatic chambers to allow identification.

The identification of some species was verified by specialists from “The Natural History Museum of London Identification Service”. The species list was then compared with information in the literature about; a) host plant range, b) herbivore distribution, c) plant organs damaged by larvae or adults, d) way of feeding. For this comparison the following definitions were adopted. The herbivore is termed monophagous if it is feeding on only one plant genus (*Heracleum*), oligophagous if it is restricted to one family (Apiaceae) and polyphagous if it is feeding on different families. Only secondary hosts of the dioecious aphids were considered to define the herbivore specificity, because the impacts on the woody primary hosts are normally negligible.

Data from previously large-scale investigations of herbivores on *H. mantegazzianum* in England, Switzerland and Slovakia (Sampson, 1990; Bürki and Nentwig, 1997; Cagan and Nentwig, 1998) supplemented our data and are included in this study. Scattered information was also gathered from 161 publications and various insect keys containing species information from Europe and the Caucasus. For information about some insect species the Russian literature was specifically researched. Two other publications on alien weeds also report on the insect fauna on native vs. invasive weed populations and give information on the feeding specificity of the herbivores. These data from Jobin et al. (1996) and Imura (2003) are compared with our own data. Statistical treatments include multiple comparisons carried out with contingency tests adjusted after Bonferroni (Howell and Games, 1974). Niche size comparisons are investigated with either two-tailed t-tests or contingency tests.

3. Results

3.1. Insect species

Overall, we gathered information about 358 insect species occurring on 16 different *Heracleum* species. Of these insects, 265 were herbivores and are used for the here presented analyses. About 162 species are herbivores on *H. mantegazzianum*, of which 123 are polyphagous or have unknown specificity. These were omitted from Table 1 but were used in the analyses. The remaining 39 monophagous and oligophagous herbivore species from *H. mantegazzianum* are presented in Table 1. They belong to four orders: Hemiptera (8 aphid

species, 4 bug species), Coleoptera (5 species among 9 are curculionids), Lepidoptera (all moths), and Diptera (half of the species belong to Agromyzidae). Among these species, the following oligophagous species were the most abundant in the examined regions: *Anuraphis subterranea*, *Cavariella* spp., *Lixus iridis*, *Agonopterix caucasella*, *Depressaria pastinacella* and *Melanogromyza heracleana*. The monophagous aphid *Paramyzus heraclei* transmits yellow spots to the plant, possibly a virus. As far as the host plant preferences are known, none of the species feeds exclusively on *H. mantegazzianum*.

3.2. Community descriptions

Fig. 1 shows the taxonomic distribution of 264 herbivorous insects in the genus *Heracleum*. Two thirds of all species belong to Hemiptera and Coleoptera but monophagous species are so far only found in Hemiptera and Diptera.

Specialist herbivore species on invasive weeds make up a significantly higher proportion of the herbivores in the native areas compared to the invaded area (Fig. 2). The insects feeding on goldenrod *Solidago altissima* L. and horsenettle *Solanum carolinense* L. were divided into generalists and specialists by Jobin et al. (1996, specialists are within genus, $n = 276$) and Imura (2003, specialists are within family, $n = 57$) respectively. The following variables (specialist/generalist, invasive/native, *H. mantegazzianum*/ *S. carolinense*/ *S. altissima*) were analysed with a $2 \times 2 \times 3$ contingency test for partial independence. The proportion of specialists is significantly depending on whether the weed is invasive or native ($p < 0.001$) and on which of the three invasive weed species is examined ($p = 0.0029$).

The proportions of each insect order found in the native area in the Caucasus compared with the invaded part of Europe is not significantly different (Fig. 3). We also compared the number of species found in a specific order on *H. mantegazzianum* with (1) the percentage of species found worldwide (Bernays, 2003) and (2) with 5610 phytophagous insects found on beneficial plants of the former USSR (data from Kryzhanovskij, 1974; Narchuk and Tryapitzin; 1981, Kuznetsov, 1999) (Fig. 3). This comparison shows that in the Caucasus and in Europe, a significantly larger proportion of species from Hemiptera are found on *H.*

mantegazzianum than expected from the world species abundance (2 x 2 contingency test, $p \ll 0.00001$). The proportion of Lepidopteran species is significantly lower on *H. mantegazzianum* in Europe and in the Caucasus than worldwide ($p \leq 0.023$) and lower than in the USSR in general ($p \leq 0.016$). Diptera and Hymenoptera are less frequent in Europe than expected from their worldwide distribution (Fig. 3).

Root borers and chewers largely belonging to Coleoptera constitute the largest feeding group (Fig. 4). Sap sucking herbivores (primarily from Hemiptera) constitute the second largest feeding group. Gall forming insects predominantly belong to Cecidomyiidae which produce galls in the leaves and the umbel, whereas leaf miners are from the Diptera order (Fig. 4).

The stem and roots are occupied with fewer species compared to the relative niche size (biomass) of these organs (Fig. 5) (t-test). On the other hand, more species are found on the leaves compared to the relative biomass of *H. mantegazzianum* in July and August. The proportion of 162 herbivorous species found in leaves, umbel, stem, and root are, however, not significantly different when the Caucasus is compared with Europe (Fig. 5) (2 x 5 contingency test, $p = 0.30$).

4. Discussion

4.1. Characteristics of the herbivore community

Strong et al. (1984) reasoned that herbivores from the pool of native species present in any region rapidly and asymptotically accumulate on introduced plants. Some recruitment of herbivorous species seems likely to occur even within the rather short time of 60-70 years. The close resemblance of the herbivorous insect associations on giant hogweed in the Caucasus compared with Europe confirms this assumption.

The first insects to colonize a new host plant are polyphagous herbivores. A low proportion of endophages is considered to be characteristic for young herbivore communities on introduced plant species, since they need to be better adapted to the chemistry and structure of the plant

(Strong et al. 1984, Frenzel and Brandl 1998). We found a higher proportion of specialized monophagous and oligophagous species in the Caucasus area compared to the invaded European countries. The same had also been also observed for other invasive plants (e.g. Jobin et al., 1996, Imura 2003). The enemy release hypothesis predicts that a larger proportion of specialist species and/or a higher density/biomass of these specialists should be found in the native Caucasus area where they would do more damage to the host plant. These three investigations of specialists insects on invasive versus native weeds have been used in this investigation, but more invasive weed species have to be studied to safely conclude that native weeds have a higher proportion of specialists. Our investigation nicely supports the first prediction and therefore endorses both the enemy release hypothesis (ERH, Keane and Crawley 2002) and the evolution of increased competitive ability hypothesis (EICA Müller-Schärer et al. 2004). Our personal assessment is, in addition, that even if the relative damage by herbivores is small in both regions, it is higher in the Caucasus area. Supporting this statement, our investigations have demonstrated that the defence system of giant hogweed is more highly developed in the native Caucasus and therefore indirectly indicates a higher herbivore load (Hattendorf 2005).

The observed over-representation of species from Hemiptera and the under-representation of Lepidoptera and Diptera has also been found previously in comparable studies (Imura, 2003; Simberloff, 2003). As a result of the Hemiptera overrepresentation, there is a high abundance of sap sucking species on *H. mantegazzianum* (fig. 4). The chewing insects form another large feeding guild (41%).

4.2. Feeding specificity

One aim of this study was to evaluate different phytophagous insect species feeding specifically on *H. mantegazzianum* and their niches in the native and the invaded areas. Considering the extensive insect collections made so far, covering a large part of the distribution range of giant hogweed, it is realistic to assume that most of the specialised herbivores may have been collected meanwhile. In the Caucasus area we have not as yet found insects exclusively feeding on *H. mantegazzianum* (Table 1), thus this result could be

called disappointing. On the other hand, in the Caucasus we found several herbivores of unknown host specificity: *Nastus fausti*, *Phytoecia boeberi*, *Otiorhynchus tartarchiani*, *Melanagromyza heracleana* (new species) and *Agonopterix caucasella* (new species). Since some of the species in these genera are known to be monophagous, they could represent potential agents for biocontrol but much more information about their ecology is needed.

4.3 Niche sizes

The biomass of the plant organs of *H. mantegazzianum* were determined and these can be considered as a representation of the relative niche size, even if a biomass determination throughout the whole growing season would be preferred to our measured biomass in July and August. These niches were found to be not equally occupied (fig. 5). Umbels have seeds with a high nutritional value and are an exposed plant organ which would favour a high herbivore load (Lawton and Schroeder 1977). On the other hand, umbels and seeds have very high furanocoumarin contents (Berenbaum 1981) and are only available during a rather short time out of the 2 or 3 years of lifespan of giant hogweed. Both would be in favour of a smaller species number (Frenzel and Brandl 1998). The roots represent a long living organ important for the plant. They should therefore be well defended (e.g. by chemical defence), and visited only by lower numbers of species, predominantly specialists. Figure 5 shows that the root and stem comprise a large proportion of the *H. mantegazzianum* biomass but only a few species were found feeding on it. Stems contain large amounts of structural compounds leading to a lower nutritional value and the observed low number of species on the stem supports this idea. Leaves are, on the other hand, easily accessible and digestible, and this is probably the reason why a significantly higher proportion of species are found on the leaves compared to their niche size.

We detected a significant difference between the proportions of insect species on each plant organ compared to the niche size (biomass). We also found a difference between the two regions in the proportion of specialists (fig. 2), but the proportion of species in each insect order and on each plant organ are not different in Europe compared to the Caucasus (fig 3 and 5). In Europe and in the Caucasus, we never observed such high herbivore densities that they

were killing the plants. Such a low damage level could be due to an intensive predation of the phytophagous insects and/or to effective plant defence mechanisms.

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Part II

Table 1: Monophagous and oligophagous herbivore species from *H. mantegazzianum* in Europe and in the Caucasus.

	Feeding specificity	Stage collected ^a	Way of feeding ^b	Plant organ ^c	Locality ^d	Source
Hemiptera						
Pentatomidae						
<i>Graphosoma lineatum</i> L.	oligo	L/A	sap sucker	umbel	EU/CAU	Wagner 1966; Jakob et al. 1998; Hansen & Hattendorf unpubl. ^f
Miridae						
<i>Orthops basal</i> Costa	oligo	A	sap sucker	stem, umbel, leaves	EU ^{OC}	Sampson 1990; Nielsen & Ravn unpubl. ^f
<i>Orthops campestris</i> L.	oligo	L/A	sap sucker	stem, umbel	EU ^{OC}	Grace & Nelson 1981; Bürki & Nentwig 1997; Hansen & Hattendorf unpubl. ^f
<i>Orthops kalmii</i> L.	oligo	A	sap sucker	stem, umbel	EU ^{OC}	Bürki & Nentwig 1997; Jakob et al. 1998; Nielsen & Ravn unpubl. ^f
Aphididae						
<i>Anuraphis subterranea</i> Walker	oligo	L/A	sap sucker	Leaf envelope	EU/CAU	Bürki & Nentwig 1997; Hansen & Hattendorf unpubl. ^f
<i>Cavariella aegopodii</i> Scopoli	oligo	L/A	disease transmitter, sap sucker	Stem, umbel, leaves	EU/CAU	Holman 1991; Sampson 1990 & 1994; Nielsen & Ravn unpubl. ^f
<i>Cavariella aquatica</i> Gillette & Bragg	oligo	L/A	sap sucker	stem, umbel, leaves	CAU	Hansen & Hattendorf unpubl. ^f
<i>Cavariella pastinacea</i> L.	oligo	L/A	disease transmitter, sap sucker	stem, umbel, leaves	EU/CAU	Sampson 1990 & 1994; Holman 1991; Bürki & Nentwig 1997; Hansen & Hattendorf unpubl. ^f
<i>Cavariella theobaldi</i> Gillette & Bragg	oligo	L/A	disease transmitter, sap sucker	stem, umbel, leaves	EU/CAU	Sampson 1990; Nielsen & Ravn unpubl. ^f ; Hansen & Hattendorf unpubl. ^f
<i>Dysaphis lauberti</i> Börner	oligo	L/A	sap sucker	stem, umbel, leaves	EU/CAU	Hansen & Hattendorf unpubl. ^f
<i>Dysaphis newskyi</i> newskyi Börner	mono	L/A	sap sucker	stem, umbel, leaves	EU ^{OC}	Heie 1992; Hansen & Hattendorf unpubl. ^f
<i>Paramyzus heraclei</i> Börner	mono	L/A	disease transmitter, sap sucker	leaves	EU/CAU	Sampson 1990; Heie 1994; Hansen & Hattendorf unpubl. ^f
Coleoptera						
Cerambycidae						
<i>Phytoecia boeberi</i> Ganglbauer		A		stem, leaves	CAU	Hansen & Hattendorf unpubl. ^f
<i>Phytoecia nigripes</i> Voet	oligo	L/A	root borer, stem borer,	stem, root	CAU ^{OE}	Koch 1992; Hansen & Hattendorf unpubl. ^f

	Feeding specificity	Stage collected ^a	Way of feeding ^b	Plant organ ^c	Locality ^d	Source
Chrysomelidae						
<i>Chrysochloa alpestris</i> Schummel	oligo	L/A	leaf chewer	leaves	EU	Cagán & Nentwig 1998
Curculionidae						
<i>Calosirus apicalis</i> Gyllenhal	oligo	L/A	leaf chewer	leaves, root	EU	Koch 1992; Jakob et al. 1998
<i>Liophloeus tessulatus</i> Müller	oligo	L/A	root borer, leaf chewer	stem, leaves, root	EU ^{OC}	Bürki & Nentwig 1997; Cagán & Nentwig 1998; Hansen & Hattendorf unpubl. ^f
<i>Liophloeus lentus</i> Germar	oligo	A	root borer, leaf chewer	root, leaves	EU	Cagán & Nentwig 1998
<i>Lixus iridis</i> Olivier	oligo	L/A	stem borer, leaf chewer	stem, leaves	EU/CAU	Cagán & Nentwig 1998; Hansen & Hattendorf unpubl. ^f
<i>Otiorhynchus tatarchani</i> Reitter		A	root borer, leaf chewer	root, leaves	CAU	Hansen & Hattendorf unpubl. ^f
<i>Nastus fausti</i> Reitter		L/A	root borer, leaf chewer	root, stem, leaves	CAU	Hansen & Hattendorf unpubl. ^f
Lepidoptera						
Epermeniidae						
<i>Epermenia</i> <i>chaerophyllella</i> Goeze	oligo	E/L/P	leaf miner	leaves	EU ^{OC}	Sampson 1990; Emmet 1996
<i>Epermenia illigerella</i> Hubner	oligo	L/P/A	leaf chewer	leaves	EU ^{OC}	Cagán & Nentwig 1998
<i>Phaulernis dentella</i> Zeller	oligo	E/L/P	chewing	umbel, leaves	EU	Sampson 1990
Noctuidae						
<i>Dasypolia templi</i> Thunberg	oligo	L	chewing	root, stem, umbel, leaves	CAU ^{OE}	Seppänen 1970; Hansen & Hattendorf unpubl. ^f
Oecophoridae						
<i>Depressaria pastinacella</i> Duponchel	oligo	L/P	chewing	umbel	EU/CAU	Sampson 1990; Bürki & Nentwig 1997; Hansen & Hattendorf unpubl. ^f
<i>Agonopterix heracleana</i> L.	oligo	L	leaf roller, umbel chewing	leaves	EU/CAU	Emmet 1979; Sampson 1990; Hansen & Hattendorf unpubl. ^f
<i>Agonopterix caucasella</i> Zlobin (new species)		L/P	Umbel chewing			Nielsen & Ravn unpubl. ^f
Tortricidae						
<i>Cydia gallicana</i> Guenée	oligo	L	chewing	umbel	EU ^{OC}	Emmet 1979; Sampson 1990
Diptera						
Tephritidae						
<i>Euleia heraclei</i> L.	oligo	L	leaf miner, chewing	leaves	EU ^{OC}	Sampson 1990; Cagán & Nentwig 1998; Hansen & Hattendorf unpubl. ^f

	Feeding specificity	Stage collected ^a	Way of feeding ^b	Plant organ ^c	Locality ^d	Source
Agromyzidae						
<i>Melanagromyza angeliciphaga</i> Spencer	oligo	L/P/A	stem borer	stem	EU/CAU	Spencer 1972; Bürki & Nentwig 1997; Jakob et al. 1998; Hansen & Hattendorf unpubl. ^f
<i>Melanagromyza heracleana</i> Zlobin (new species)	oligo	L/P/A	stem borer	stem	CAU	Hansen & Hattendorf unpubl. ^f
<i>Phytomyza spondylii</i> Goureau	oligo	L/P	leaf miner	leaves	EU ^{OC}	Ashwood-Smith et al. 1984; Bürki & Nentwig 1997; Sampson 1990; Nielsen & Ravn unpubl. ^f
<i>Phytomyza sphondyliivora</i> Spencer	oligo	L	leaf miner	leaves	EU/CAU	Spencer 1972; Nielsen & Ravn unpubl. ^f ;
<i>Pegomya versicolor</i> Meigen ^e	mono	L	leaf miner	leaves	EU	Bei-Bienko et al. 1989; Sheppard 1991
Cecidomyiidae						
<i>Contarinia heraclei</i> Rübsaamen ^e	mono	L	chewing	leaves	EU	Bei-Bienko et al. 1989
<i>Contarinia nikolayi</i> Rübsaamen ^e	mono	L	chewing	umbel	EU	Bei-Bienko et al. 1989; Nijveldt 1995; Sampson 1990
<i>Macrolabis heraclei</i> Kaltenbach ^e	mono	L	gall former	leaves	EU ^{OC}	Bei-Bienko et al. 1989; Nijveldt 1995
Psilidae						
<i>Psila rosae</i> Fabricius	oligo	L	root borer	root	EU ^{OC}	Hardmann & Ellis 1982; Nielsen & Ravn unpubl. ^f ; Hansen & Hattendorf unpubl. ^f

^a stages collected: E = eggs, L = larvae, P = pupae, A = adults.

^b mono = feeds only on *Heracleum* spp., oligo = feeds on Apiaceae, poly = feeds on several plant families.

^c plant organ: umbel = feeding on seeds and flower stalks but not on pollen and nectar.

^d Locality: The locality, where the species is found, is noted as Europe = EU and Caucasus = CAU. EU/CAU = found in both regions. EU does not mean that this species is not occurring in the Caucasus, but just that it had not been found so far on *H. mantegazzianum* in the Caucasus.

^{OC} = Occurs in Caucasus but so far not found on *H. mantegazzianum*.

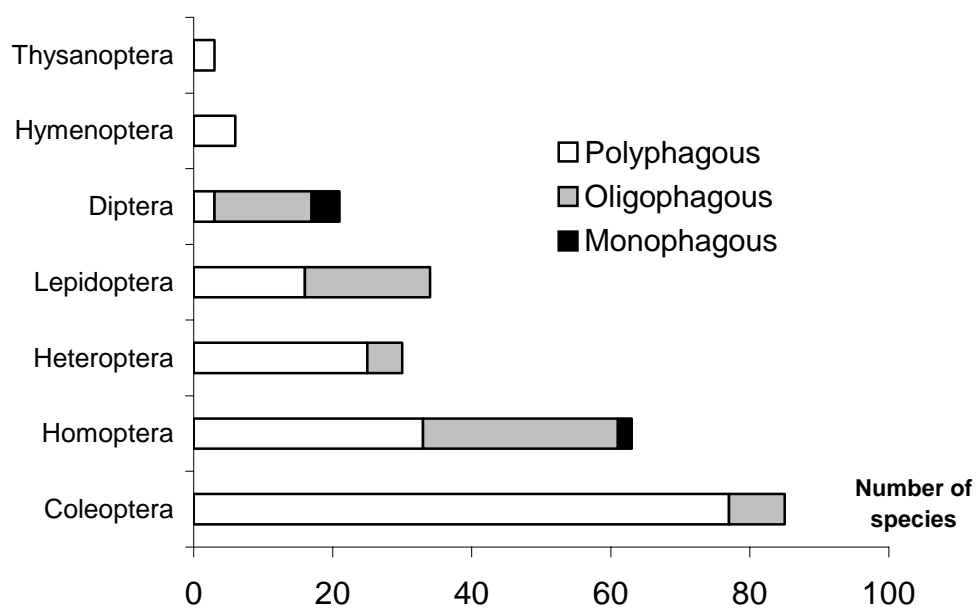
^{OE} = Occurs in Europe but so far not found on *H. mantegazzianum*.

^e Found only on other *Heracleum* species than *H. mantegazzianum*.

^f Collected during field trips in 2002 in the Caucasus, unpublished.

Part II

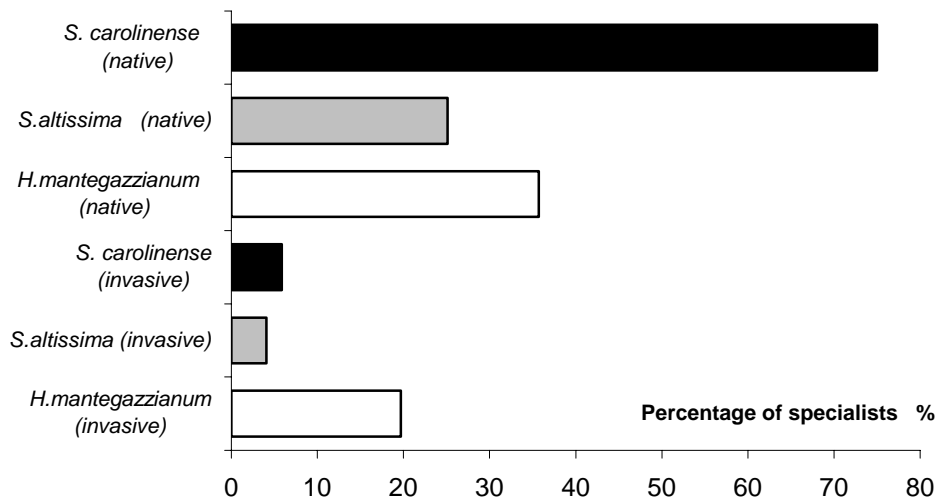
Fig. 1: Taxonomic origin of 264 herbivorous species found on *Heracleum spp.* Most species are polyphagous. Oligophagous herbivores are restricted to 5 taxonomic groups, monophagous are only found in Hemiptera and Diptera.



Part II

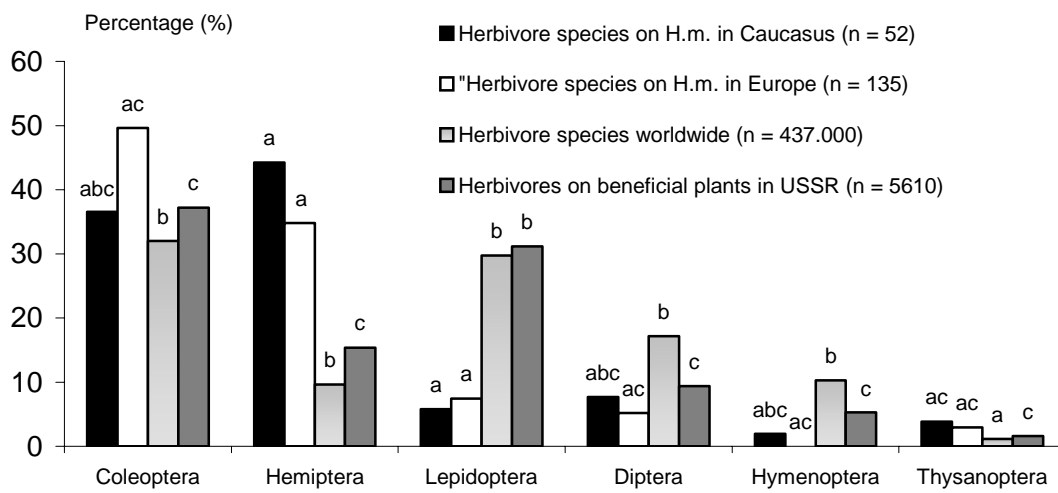
Part II

Fig. 2: Specialist herbivore species on invasive weeds make up a significantly higher proportion of the herbivores in the native areas compared to the invaded areas. Data for goldenrod *Solidago altissima* L. from Jobin et al. (1996, n = 276), data for horsenettle *Solanum carolinense* L. from Imura (2003, n = 57). The proportion of specialists is dependent as to whether the weed is invasive or native ($p < 0.001$) and different for each of the three invasive weed species ($p = 0.0029$).



Part II

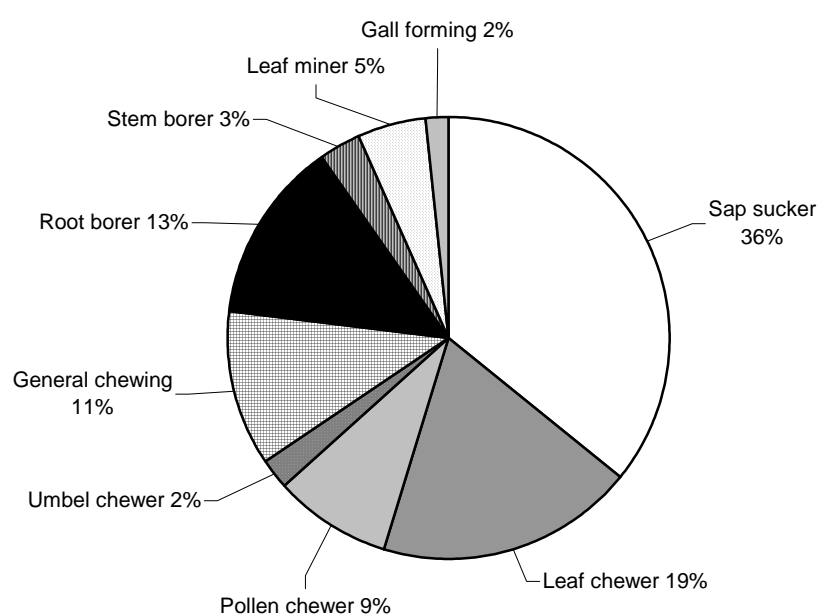
Fig. 3: Percentage of herbivores on *H. mantegazzianum* belonging to different insect order. Different letters above the columns for the same insect order refer to a significant difference in a 2 x 2 contingency test ($p < 0.05$). Data on the worldwide number of herbivore species according to Bernays (2003); data on herbivores on beneficial plants in the former USSR according to Kryzhanovskij (1974), Narchuk and Tryapitzin (1981), and Kuznetzov (1999).



Part II

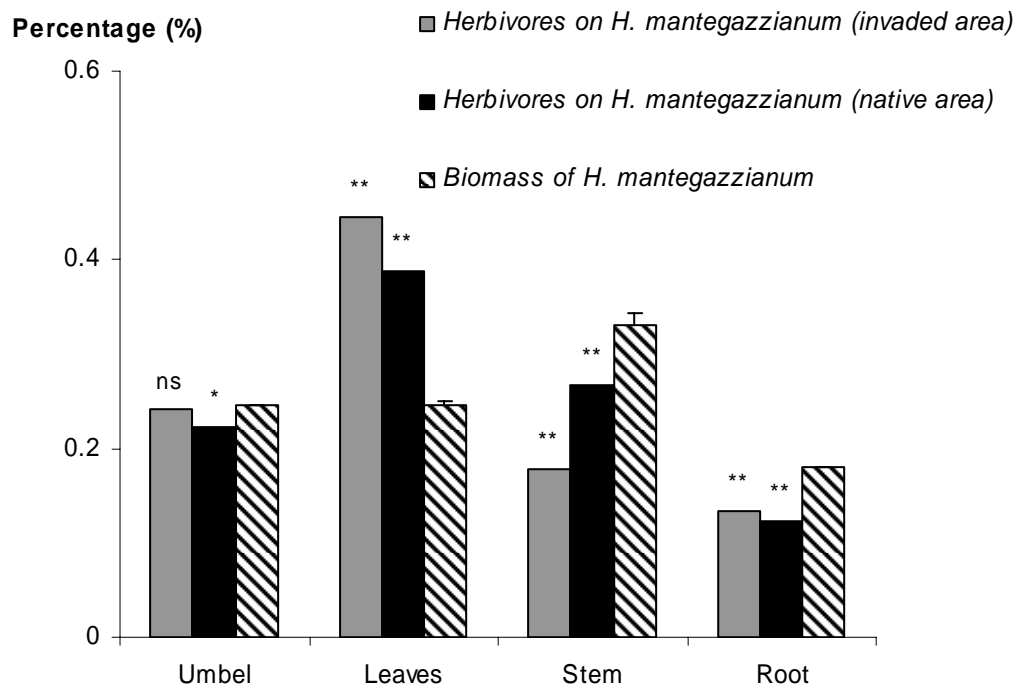
Part II

Fig. 4: The feeding habits (feeding guilds) of the 264 insect species found on *Heracleum* spp. Multiple entries are allowed as the larvae sometimes feed on different organs than the adults. Chewing insects are feeding on external plant organs and umbel chewers represent the insects chewing on the seeds or the flower stalks.



Part II

Fig. 5: The percentage of insect species belonging to each plant feeding niches in Europe and in the Caucasus is compared with the relative biomass \pm SE of these plant organs (relative niche size). No significant difference is found between the proportions of insect species on *H. mantegazzianum* in the native areas ($n = 53$) compared with the invaded areas ($n = 133$). An asterisk indicate a significant difference between proportion of species and the proportion of biomass belonging to the particular plant organ (ns = non significant).



Part III

Two plant responses induced by aphids on the invasive Giant Hogweed *Heracleum mantegazzianum*

Two plant responses induced by aphids on the invasive Giant Hogweed *Heracleum mantegazzianum*

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Abstract. Field experiments were performed to examine different responses by Giant hogweed *Heracleum mantegazzianum* and the changes in ant behaviour, elicited by the presence of aphids *Dysaphis lauberti* to test some hypotheses explaining why ants (e.g. *Lasius niger*) tend and construct soil shelters above trophobionts on this plant.

H. mantegazzianum's chemical defence of furanocoumarins is well described. We investigated two other defence mechanisms induced by aphids. Aphids induced the plant defence of *H. mantegazzianum* directly, by creating longer trichomes with higher densities on the developing petioles. Previous studies showed that trophobionts were indirectly responsible for increased growth of *H. mantegazzianum*, but non-myrmecophilic aphids (*Cavariella theobaldi* and *Paramyzus heraclei*) were negatively correlated with plant growth (Hansen et al. 2005A). By placing the myrmecophilic aphids *D. lauberti* inside the leaf envelope, the ants were attracted and induced to construct soil-shelters. Ants are known to protect plants from herbivores which is an example of indirect plant defence. Plants treated with other ant attractants like honey solution, proteins or dead aphids, did not evoke the shelter building behaviour. Ant-made soil-shelters changed the microclimatic conditions by increasing the humidity and inhibiting aphidophagous predators (Coccinellidae) from reaching the aphid colony. The 3 plant defence systems are a huge barrier for most herbivores to overcome, and might explain why so few specialised phytophagous insects have been found on this invasive weed and why it appears so fit in both native and invaded regions.

Key words: Induced resistance, soil shelters, ants, environmental refugia, humidity, microclimate, *Lasius niger*, *Myrmica rubra*, *Myrmica ruginodis*, *Dysaphis lauberti*, plant defence, trichomes, herbivory, secondary outposts, outstations, cowsheds, arcades, trophobionts, Caucasus, plant growth.

Introduction

Plant responses to herbivory

Induced resistance has been reported from over 100 plant-herbivore systems (Karban & Baldwin 1997; Agrawal 1999). Induced plant responses may involve changes in water content (Faeth 1992), plant nitrogen (Bi et al. 1997), plant secondary chemistry (Tallamy and Raupp 1991) leaf toughness (Kudo 1996) and trichome density (e.g. Pullin and Gilbert 1989; Baur et al. 1991, Dalin & Björkman 2003) or indirect defences that act through a third party (Janzen 1966, Hölldobler and Wilson 1990, Agrawal and Fordyce 2000).

Because of induced chemical defence, aphids had reduced fecundity on herbivore-damaged plants compared with control plants in the greenhouse (Stout 1979). Trichomes are also known to prevent certain leaf-chewing herbivores from feeding (Agrawal 1999). Plants with larger spines have been shown to experience reduced herbivory compared to plants with smaller spines (Karban & Baldwin 1997; Traw & Dawson 2002). Pubescence has been reported beneficial for cotton plants by reducing the number of thrips (Schuster and Calderon 1986). The leaf beetle *Phratora vulgatissima* L. can increase the trichome densities by feeding on *Salix cinerea* L. (Dalin & Björkman 2003).

Some ants are said to have entered into trophobiosis with the homopterans who are providing the ants with food (honeydew) indirectly from the plant (Hölldobler and Wilson 1990, Itino et al. 2001). Plants bearing ant-Homoptera associations might experience reduced herbivore damage (Hansen et al. 2005A), as plants bearing extrafloral nectaries do, because of the ant activities (Carroll and Janzen, 1973). Honeydew is known to provide a large percentage of the energy budget for ant species (Horstmann 1974 and 1982, Skinner 1980), in return the ants tend the myrmecophilic aphids in many ways: construction of shelters, transport of individuals, removal of honeydew to prevent fungal diseases and aggressive defence. *Lasius niger* L. has previously been shown to repel adult coccinellids quickly from aphid aggregations. They carry off small coccinellid larvae (El-Ziady and Kennedy, 1956) and have a strong positive effect on the growth and speed of maturing of the aphid *Aphis fabae* Scopoli (El-Ziady 1960). Indirect plant defences like tropical “ant-plants” have developed in many

taxa and suggest that protected plant cavities are highly beneficial for the inhabitants and important in the evolution of ant-plant mutualisms (Jolivet 1996).

The main aim of this paper is to investigate species from different trophic levels, by examining some of the interactions between giant hogweed (*Heracleum mantegazzianum*) the herbivores and the often encountered ants. We primarily focus on the effect of aphids at the stem basis, on the ant behaviour and on the plant defences.

H. mantegazzianum's response to herbivory

H. mantegazzianum have three described defence systems against herbivores, one chemical one mechanical and the indirect ant defence (Hansen et al. 2005A). The chemical defence system exists in all plant organs and consists of furanocoumarins (Knudsen 1983, Pira et al. 1989). The toxicity of the furanocoumarins is increased in the presence of ultraviolet light which catalyzes cross-linkage of the pyrimidine bases in the DNA strands (Berenbaum, 1978).

The second defence system of *H. mantegazzianum* consists of one or two rows of tiny rigid trichomes on the edges of the leaves on the leaf undersides and along the stems. Trichomes have been shown to be a costly defence (Elle et al. 1999) and it is beneficial to reduce such cost as much as possible, e.g. by inducible defence systems. Induced defence systems are found more often on fast growing and long living plants (Karban and Balwin 1997). *H. mantegazzianum* must be considered a fast growing plant which is perennial and predominantly attacked by generalists (Hansen et al. 2005B). We may therefore expect that this plant has developed an inducible defence system. Previous investigations demonstrated that giant hogweeds trichome defence is less developed in the invaded regions (Hattendorf 2005), indicating a variable defence and consequently a variable herbivore load (Zangerl and Rutledge 1996).

The purpose of the soil shelters

The ant building behaviour of covers has been described for many ant families (Hölldobler and Wilson 1990), and during our 11 weeks in the Caucasus we also observed soil-shelters

built by ants above other aphids on other plants too (Hansen 2005). Anderson and McShea (2001) reviewed these adaptive structures (other than primary nests) built by ants over above food sources or roadways. Benson (1985) claimed that many tropical ants protect their honeydew sources by building plant-fibre shelters to extend their caring effort into the rainy periods. E.g. the silk shelters built around coccids (Homoptera) by *Oecophylla longinoda* Latreille (Way 1963). Food resources such as extrafloral nectaries can sometimes be covered (Beckmann and Stucky 1981). It has moreover been suggested that shelters may exclude predators and parasitoids (Way 1954, Gullan 1997). A domatium is defined as a natural hollow plant cavity sheltering insects beneficial for the plant (Anderson and McShea 2001), and the leaf envelope on *H. mantegazzianum* are considered to be such one (Hansen et al. 2005A). Artificial domatia have shown to increase the number of predaceous bugs and therefore benefiting the plant (Agrawal et al. 2000) by reducing the number of herbivores. Aphids, spider mites and whiteflies decreased in numbers and the plant defence strategies were induced. The predaceous bug eggs outside a domatium had a parasitism rate of 32 %, compared with zero parasitized eggs inside the domatium. These domatia were mainly housing predators and rarely herbivores (Agrawal et al. 2000).

During investigations 2002 of three localities in the Russian Caucasus, 53 % of the randomly selected giant hogweed plants (n = 70) had aphids (*Dysaphis lauberti* Börner or *Anuraphis subterranea* Walker) inside the leaf envelope (Hansen 2005). In 2002-2003 *L. niger* constructed 57 % (n = 166) of the observed shelters, while *M. rubra* and *M. ruginodis* constructed 39 % of the shelters and 4 % had a uncertain origin. For this system, the following hypotheses may explain why and what triggers *L. niger*, *M. ruginodis*, or *M. rubra* to construct soil shelters above *D. lauberti*:

1. Only when ants have already created the soil-shelter, the aphids will enter.
2. Soil shelters are a part of the ants primary nest.
3. Soil shelters protect aphids against predators or parasitoids (Way 1954, Gullan 1997).
4. Soil shelters improve soil microclimatic conditions for either aphids or ants (humidity and temperature) (Agrawal et al. 2000, Anderson and McShea 2001).

The shape and size of shelters are rarely described, and the details of the mechanisms that induce the constructions of the soil-structures are also poorly understood (reviewed by Anderson and McShea 2001). In this paper we want to describe and investigate these mechanisms, and to examine the interactions between plants-aphids-ants, by answering the following questions, derived from the above hypothesis: a) Do aphids or food resources induce soil shelter construction?, b) Do aphids induce trichome development?, c) Do soil shelters block for the predators (coccinellids) of the trophobionts?, d) Do soil shelters improve microclimate?

Materials & Methods

Shelter induction

This experiment was set up June 2004 in Pregradnaja at the northern slope of the Caucasus in Russia (N 43° 54' 26'' E 041° 17' 03''). The habitat is 2 ha large and inhabited by a grass and herb community surrounded by forest, which in former times served as agricultural fields. The 150 *H. mantegazzianum* plants included in the shelter induction experiment were initially lacking ant constructed soil shelters. Following treatments were applied to two-year-old plants; 1) Ten apterous viviparous females of *D. lauberti* were transferred into the leaf envelope; 2) 20 dead individuals of *D. lauberti* (squashed) were placed in the leaf envelope; 3) a honeydew source was simulated with a saturated liquid solution of bee honey in a glass cylinder covered with a cotton plug was attached to the leaf envelope; 4) protein (tuna) placed in a cylinder was attached to the leaf envelope; 5) no treatment (control). The presence of soil shelter, the shelter size, aphid numbers inside the shelter and the ant activity (number of ants near the leaf envelope during one minute), was determined in the end of the experimental period of 40 days, but also collected the previous year 2003, at the same locality.

Apart from *Dysaphis lauberti*, other aphid species were feeding on *H. mantegazzianum* in low densities at both visits in 2004. These species were *Paramyzus heraclei* Börner, *Cavariella theobaldi* Gillette & Bragg and *Anuraphis subterranea*. *P. heraclei* and *C. theobaldi* feeds only on leaves and flowers (Hansen et al. 2005B). Only *D. lauberti* was present in the start of the experiment, *A. subterranea* arrived later, and invaded our experimental plants in low frequencies and densities. *A. subterranea* is feeding inside the same leaf envelopes as *D.*

lauberti and they are both myrmecophilic. If plants were infested by aphids that were not a part of the treatment or if they disappeared from the treated plants, they were discarded from the experiment (39 plants).

Predator defence

Ladybirds like *Adalia bipunctata* L. and *Coccinella 7-punctata* L. (Coccinellidae) were regularly found feeding on aphids on *H. mantegazzianum* under natural conditions in both the Caucasus and Europe. To examine if the soil shelter prevents such predators from entering the leaf envelope and feeding on the aphids, an experiment was set up in the institute's garden in Bern, Switzerland N 46° 57' 07'' E 007° 25' 41'' on planted *H. mantegazzianum* plants. One hundred *A. bipunctata* larvae and 79 adults were released (9 beetles per plant), on the stem 3 cm below the leaf envelope, on plants with or without ant-made soil-shelters. The ladybirds, *Adalia bipunctata* were obtained from Andermatt Biocontrol AG, Switzerland. The number of individuals entering the leaf envelope, when they approached the leaf envelope, within 5 minutes of foraging, was determined for 10 plants with ant-made soil shelters and for 10 plants without.

Microclimatic changes

The temperature and humidity in one ant made soil shelter and 5 cm above it were measured simultaneously using a pair of Vaisala humidity probes and two thermocouples connected to a data logger (Grant Instruments Cambridge Ltd). The measurement started August 27th 2003 at 17.00 o'clock, after one week of no rain, and ended 17.00 o'clock a day later (28th) in the institute's garden in Bern. Measuring intervals were 60 minutes and the shelter, thermocouples and humidity probes, were never exposed to the direct sun light. To achieve this the sun had to be blocked for 3 hours with an umbrella.

Trichome induction

This experiment was also set up June 2004 in Pregradnaja, Russia. The 120 two-year-old *H. mantegazzianum* plants, included in the shelter induction experiment, were initially lacking ant constructed soil shelters. The leaf envelopes were either treated with ten apterous

viviparous females of *D. lauberti* or not treated as control. The following parameters were measured before and after an experimental period of 40 days: petiole length, petiole diameter, length and density of the trichomes (within an area of 1.1 cm²) on the largest petiole stem and on the youngest petiole stem. Only the plants treated with aphids were named induced if there were aphids inside the shelter at the end of the experiment, and if aphids invaded the control plants the results were discarded as well. If plants had a considerable number of other herbivore insects on the leaves and stems (more than 5 individuals during 2 minutes of observation at the end of the experimental period), the result were discarded from the experiment. In total, 46 plants were discarded due to the reasons above. Thirty-six of the remaining 74 plants had new petiole stems less than 30 cm long, and constituted our experiment “young petioles”.

Statistics

To identify which treatment induced the ants to build shelters, 2x2 contingency tests were performed, by comparing with the control. The proportion of predators entering the leaf envelope with or without shelter was also tested with 2x2 contingency tests. Induction of trichome densities and lengths were tested with t-tests. If the trichome density was significant influenced by petiole diameter, then the results was submitted to an ANOVA, where the stem diameter set as a covariate, in order to make the trichome density independent of petiole diameter. The relationship between the aphid numbers, the ant activity and the size of the soil-shelter was assessed by means of a Spearman rank correlation. The analyses were carried out using the SPSS 12.0.1 statistical package.

Results

Shelter induction

Soil shelters are constructed by ants, on top of the leaf envelope, at the stem basis of 2-3 year old *H. mantegazzianum* plants, to cover colonies of aphids. Later in the season these soil shelters can surround the whole stem basis with numerous aphids and comprise a surface area of more than 200 cm². Only live individuals of *D. lauberti* induced the ants to build the soil shelters (fig. 2). 39 plants were discarded because aphids invaded or aphids disappeared from

the treated plants. Table 1 demonstrates that there is a highly significant correlation between aphid numbers, ant activity and the size of soil shelter which again indicates that aphids induce the ants to build larger soil shelters. In July 2004, in Pregradnaja Caucasus, the soil shelters had a mean surface area \pm SE of $7 \pm 1 \text{ cm}^2$ ($n = 25$, including discarded observations) but in July of the previous year they were $18 \pm 2 \text{ cm}^2$ ($n = 88$). In 2004 the average ant activity (\pm SE) was 3.5 ± 0.2 ants per minute in a 10 cm radius around the stem. At 15 % of the plants no ants were observed within 1 minute, both before and after the experiment. At the end of the experiment, the final average number of aphids inside the leaf envelopes with soil shelters \pm SE was 71 ± 13 individuals (range: 1 - 400).

Predator defence

The results show that the soil shelters repel aphidophagous insects (fig. 3) but also shows that shelters are not 100 % effective in repelling and preventing predators from entering the aphid colonies. During our observations in the Caucasus, we only rarely (3 out of several hundred observations) found syrphid and coccinellid larvae feeding on aphids beneath the soil shelters.

Microclimatic changes

The temperature in the *L. niger* soil shelters was reduced inside the shelter compared to outside between 10.00 and 21.00 o'clock. Maximal difference of 4.6°C was observed in the afternoon. Temperature was not reduced during the night (10.00 and 21.00 o'clock). In contrast, humidity was constantly higher inside the soil shelter during the 24 hours (fig. 4).

Trichome induction

The two-year-old plants of *H. mantegazzianum* had one or two rows of tiny rigid trichomes on the edges of their leaves (length range $0.5\text{-}3 \mu\text{m}$), extending from the smallest veins on the underside of the leaves and further on to the petiole stems and on the main stem. The whole plant is covered with trichomes except in the leaf envelope where *D. lauberti* is situated, and the upper side of the leaves, where no trichomes are present. The pooled average trichome length on older petioles \pm SE is $3.86 \pm 0.10 \text{ mm}$ (fig. 6) (range 2 - 8 mm).

By placing aphids inside the envelope, the trichome densities and lengths were not changed significantly on the older petiole stems ($p > 0.61$) (fig. 5-6). In contrast are the trichome densities on the developing petioles inducible (ANOVA, $F = 36.8$, $p < 0.001$, $n = 36$). The stem diameter had a significant impact on the trichome density for the developing (new) petioles and were included in the ANOVA as a covariable. The trichome lengths also increased significantly on the new developing petioles with aphids ($p < 0.001$) (fig. 5-6), but were independent of stem diameter ($p = 0.90$).

Discussion

Shelter induction

Only the 2 and 3 year old plants have an envelope open enough for ants and aphids to enter and that is probably why soil shelters are never observed on one year old plants. Our experiments with two-year-old plants clearly demonstrated that the presence of aphids induced the ants to build the soil shelters (table 1, fig. 2). *L. niger* build the soil shelters only around aphid colonies on *H. mantegazzianum* and therefore confirm observations that they build arcades and above ground tunnels, that are not just are a part of the ants primary nest (Gösswald 1985, Anderson & McShea 2001). Therefore, we reject our two first hypotheses. However not all plants treated with aphids lead to the construction of soil shelters. The explanations could be that; a) the ants never discovered the aphids and obligate myrmecophilic aphids have a high mortality if they are not immediately tended and if the honeydew is not removed from the narrow leaf envelopes; b) some plants have less nutrients in the sap than needed to support aphid growth (Strong et al. 1990); c) the ant colony decides not to protect the aphids with a soil shelter, when they have a surplus of carbohydrates and do not need additional honeydew. Hölldobler and Wilson (1990) claimed that aphids occasionally produce large surpluses of honeydew. A few other explanations are possible, although the most credible under these conditions seems to be the first.

The construction of soil shelters supports the hypothesis that a mutualistic relationship exists between ants and aphids. Ant made soil shelters were shown to be of great importance to plants, aphids, and ants (Hansen et al. 2005A). Our observations suggest that another aphid

Anuraphis subterranea, from a different genera, elicit the same soil shelter building behaviour by the ants on *H. mantegazzianum*, as *D. lauberti* does. At least three species of ants, belonging to two different subfamilies (Myrmicinae and Formicinae), build shelters. The ants construction behaviour is similar and widespread, in both our and other studies, and also the constructions have a similar appearance and therefore it supports the hypothesis of homogenetic evolution (Anderson and McShea 2001). Only living aphids induce shelter building behaviour (fig. 2).

Soil shelters repel aphidophagous insects

For a coccinellid to discover an aphid colony on giant hogweed, the critical point is the actual contact to the aphid colony, since they do not detect aphids from a distance (Majerus and Kearns 1989). Soil shelters prevent this physical encounter and block predators (both larvae and adult ladybirds, fig. 3-4) from entering envelopes with aphids. This therefore supports our third hypothesis. Since ants benefit from the mutualistic relationship with aphids, it explains also why ants construct soil shelters.

Microclimatic changes

Our results support the fourth hypothesis that soil shelters improve the microclimatic conditions. The humidity is clearly elevated and temperature is slightly reduced during the daytime inside a soil shelter of *L. niger* (fig. 4). Ants and aphids profit from this microclimate. If relative humidity is higher inside the shelters and the temperature is lower during the summer period (fig. 4), it reduces their risk of desiccation (Prosser 1973, Anderson and McShea 2001). It may also reduce energy expenses because thermal overheating is avoided (Heinrich 2003) and a shelter will prolong the period where ants are tending aphids because ants avoid foraging in rain. A similar investigation of secondary nests was made for the desert thatch ant *Formica obscuripes* Forel (McIver and Steen 1994). Their nest structures is situated below the soil surface in close proximity to the root of the Sagebrush, *Artemisia tridentata* Nutt. (Asteraceae). It was in average 7 °C cooler in the secondary nest, than 25 cm above the nest. Consequently such structures can be considered as the ants external organ, in the concept of the “extended organism” (Turner 1999).

Shelter functions

We never found ant-progeny beneath the soil shelters (Hansen 2005), and because soil-shelters are built specifically around aphid colonies, we reject our first hypothesis. Shelters are not primary ants-nests, but play an important role in the interactions between the ants and aphids. We accept our 3rd and 4th hypothesis: Shelters change the microclimatic environment (fig. 4), and blocks a high proportion of aphid predators (fig. 3). Additional 4 hypotheses can also explain why ants construct the soil-shelters: They give UV-light protection. The plant sap contains furanocoumarins (Berenbaum 1978; Berenbaum and Feeny 1981) which in combination with UV light is toxic, and ants and aphids perform better when they are protected from sunlight. Shelters certainly blocks UV-light, but it is necessary to test whether this really is advantageous for aphids or ants. Soil shelters prevent the leaf envelope from getting flooded during rain. Shelters clearly absorbs rainwater, but it should also be tested whether this is a beneficial feature. It is known that rain can dislodge aphids from the plant, and ants are also known to reduce their foraging under bad weather conditions (Way 1963, Benson 1985). The shelter can be seen as a prison (pen), sealed off to prevent aphids from escaping (Anderson and McShea 2001). This idea is supported by the fact that wings of the alate aphids very often were crippled during our investigations. This biting behaviour by the guardian ants had also been described for *L. niger* by Mordvilko (1894) or the aphids are exploiting the ant's behavioural repertoire, by simulating the recognition cues and e.g. producing allomone that evoke a beneficial behavioural response. This phenomenon of exploitation of ants can for example be observed with "ant guests", slave making ants, or seed dispersal by myrmecochory (Hölldobler and Wilson 1990).

Trichome induction

Plant trichomes are normally considered as plants defence against herbivorous insects (Southwood 1986, Valverde et al. 2001, Dalin and Björkman 2003). Additional explanations is that they reduce transpiration (Jordaan and Kruger 1992; Monteiro et al. 2001), or aid foraging ants in their patrolling on the plant surfaces (Hölldobler and Wilson 1990).

Developing leaves are known to be more vulnerable to herbivory than fully developed leaves (Moles and Westoby 2000), and higher trichome densities improve the survival of young leaves. Trichomes are strongly affected by plant ontogeny (Karban and Baldwin 1997) and on *H. mantegazzianum* trichomes are actually much denser on stems with small diameters. Higher trichome densities may apply to seedlings as well, where herbivory is a crucial mortality factor. This ability is inherent and not determined by the inducibility. In this study, the presence of herbivores induced the plants defence in less than 40 days. The furanocoumarins in *Pastinaca sativa* L., a close relative to *H. mantegazzianum*, are known to respond much quicker to a herbivore attack (Zangerl & Rutledge 1996; Zangerl & Berenbaum 1998). In *P. sativa* the maximal induction of furanocoumarins was achieved after 3 hours. Therefore, we conclude that the induced trichomes of *H. mantegazzianum* are a long-term defence against gradually increasing population of herbivores, such as aphids.

The treated plants had on average 71 aphids in the soil-shelter. A larger attack from herbivores could possibly even show larger trichome inductions than the ones we observed here. This inducible trichome defence system has probably primarily evolved in order to deter the non-beneficial herbivores from feeding on the stems and leaves. Hansen et al. (2005A) argued that the ants prevent non-myrmecophilic (plant damaging) aphids from feeding, when they are foraging, and this resulted in a lower metabolite loss and the observed increased growth of *H. mantegazzianum*. Additionally are the myrmecophilic aphids inside the leaf envelope inducing the trichome formation and therefore preventing settlements on the stem and leaves. The trophobionts can occasionally be regarded as zoological devices, used by the plant to attract ants, because of ants beneficial effects on the plant growth (Gullan 1997). The plant will still benefit from the increased costs of trichome production and the three partner mutualistic relationship (*A. subterranea*, *L. niger*, *H. mantegazzianum*), if those costs are smaller than the damage-costs from other herbivores (e.g. aphids on the leaves, Hansen et al. 2005A).

We predict that the three partner mutualism, described by Hansen et al. (2005A), will evolve towards a more species-specific induction of trichomes only by aphids on the leaves (the

“damaging” aphids) and not by the “beneficial” aphids from the three partner mutualistic relationship. The induced responses and the signalling pathways have sometimes been shown to react specifically to certain herbivore attacks (Agrawal 1999, Felton and Eichenseer 1999), and unless the attack by the beneficial aphids is highly correlated with attacks by pest aphids, such defence systems are disadvantageous for the plant.

A negative correlation between number of herbivores and plant size does not necessarily imply that damage is done to the plant, but perhaps a result of an activated plant defence system. For *H. mantegazzianum* this is even more problematic, since it has three plant defences that probably all are induced by aphids. When classical biocontrol against weeds is considered, it is therefore required to estimate and quantify the damage made by a potential phytophagous control agent.

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Part III

Table 1: The number of aphids in the leaf envelope (all plants who had soil shelters), the ant activity and the size of the shelter are highly correlated. Spearman rank correlation coefficient (2-tailed significance, $n = 42$, α threshold value = 0.05).

	Ant activity (individuals/min)	<i>D. lauberti</i> (numbers in leaf envelope)
Shelter size (cm ²)	0.45 (<<0.003)**	0.69 (<<0.001)**
Ant activity (individuals/min)		0.61 (<<0.001)**

Part III

Figure 1: A domatium (leaf envelope) is a hollow cavity at the stem base of 2-3 year old *Heracleum mantegazzianum* plants, sheltering colonies of aphids (*Dysaphis lauberti*) and ants *Lasius niger* (or *Myrmica spp.*). The domatium is curved inwards (see cross section) and ants construct soil shelters on top of the domatium (leaf envelope left side) when aphids are inside (fig. 2). Soil shelters blocks the entry of aphid predators like *Adalia bipunctata* (Coccinellidae) (fig. 3). When aphids feeds on the plant, it induces the plant defence and develop petioles with longer trichomes in higher densities (fig. 5-6).

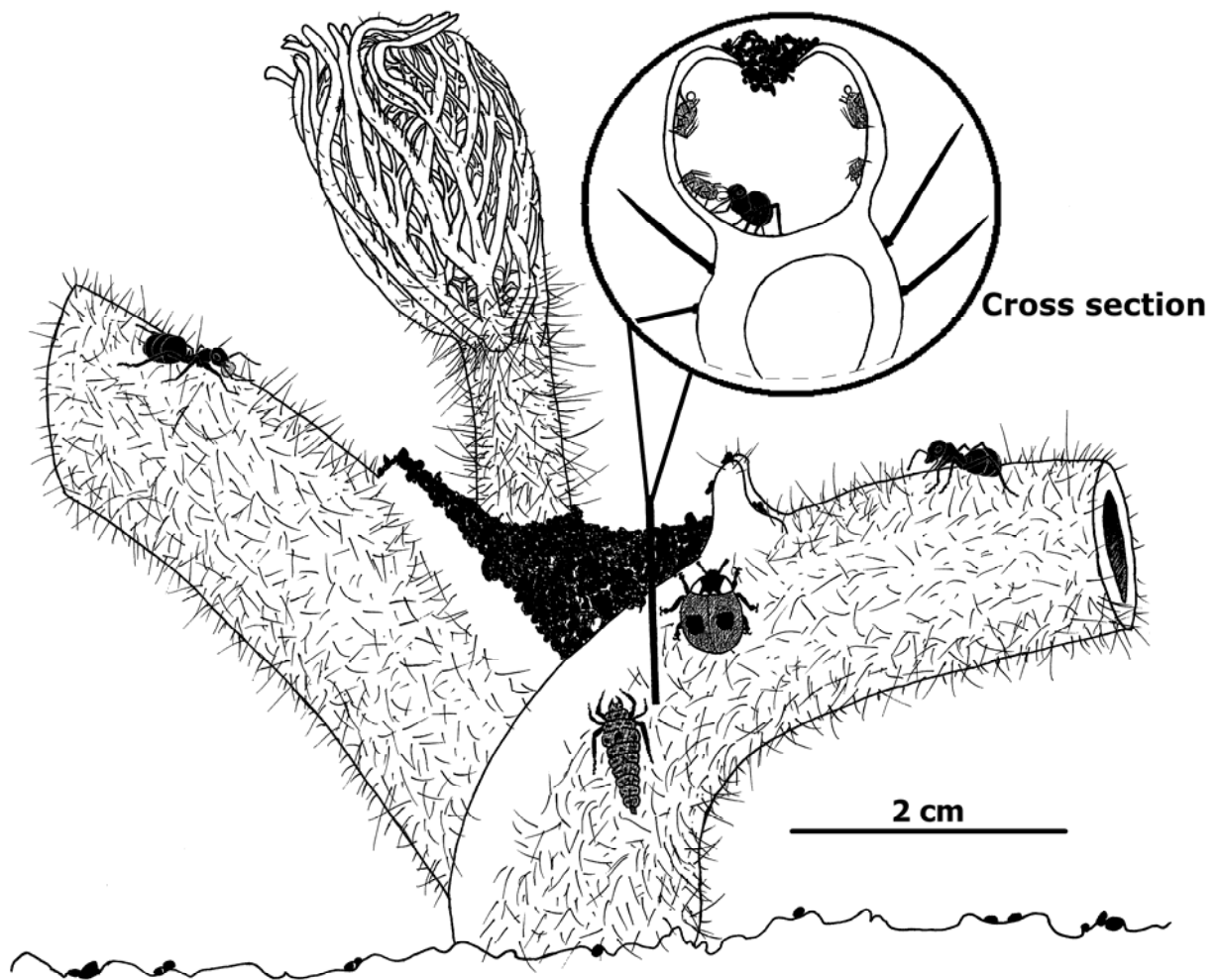
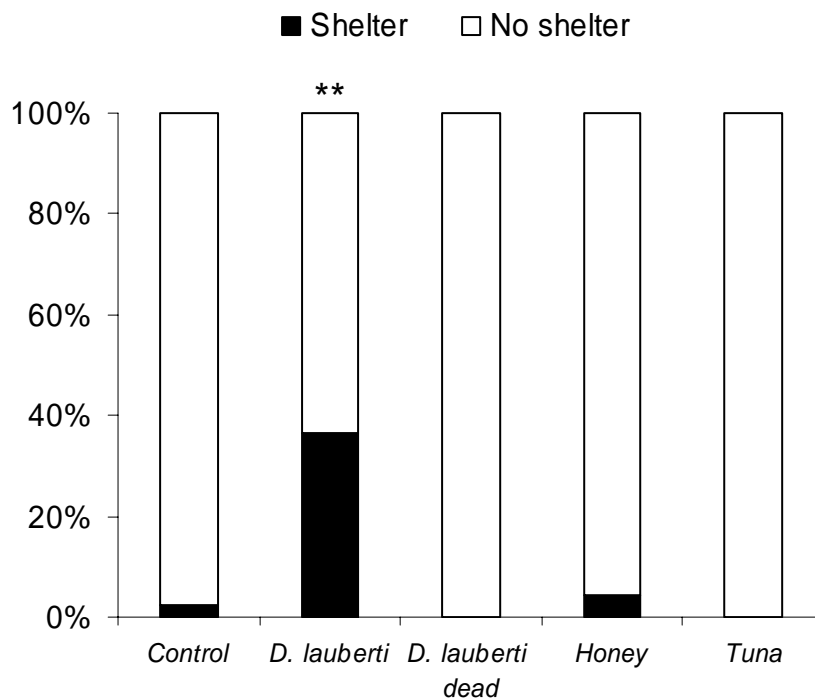


Figure 2. Proportion of treatments that induced the ants to build soil shelters. Following treatments were applied to the leaf envelopes of *H. mantegazzianum*: a) 10 aphids (*D. lauberti* fourth instars), b) 20 dead aphids (*D. lauberti*), c) water solution saturated with honey, d) protein source (tuna). Placing living aphids inside the leaf envelope induced the ants to build soil shelters around the aphids $p = 0.00015$, but none of the other treatments induced this behaviour ($p > 0.21$, $n = 111$).



Part III

Figure 3: Percentage of *Adalia bipunctata* larvae and adults entering the envelope with or without the ant made soil shelter. Two times two contingency test with one fixed margin for larvae: $p < 0.001$ ($n = 100$), and for adults, $p < 0.05$ ($n = 79$).

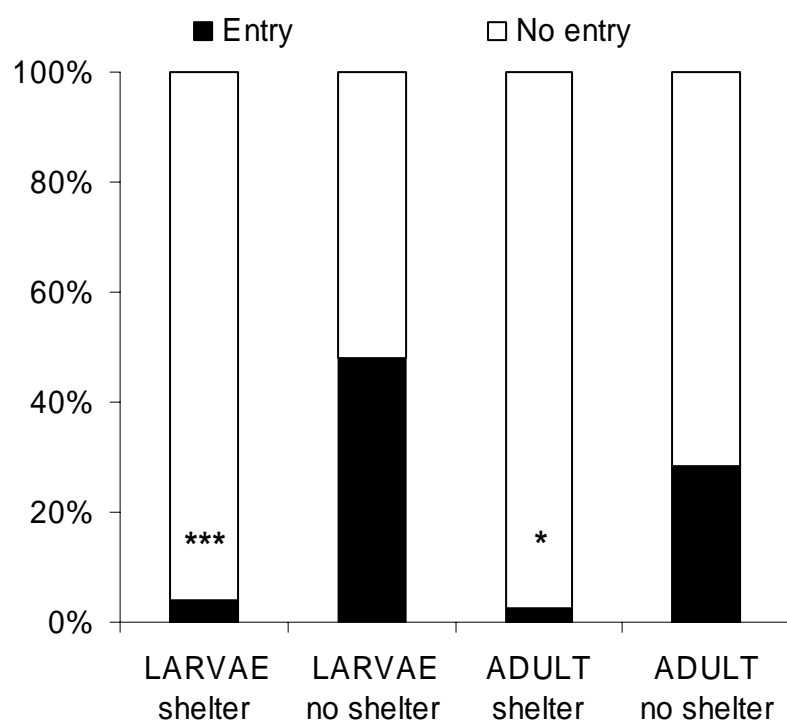


Figure 4: Effect of the ant made soil shelters on humidity and temperature. Measurements were made simultaneously during one day (27 – 28th August, 2003), after one week of no rain. The measurements outside the soil shelter were made 5 cm above it and never receiving direct sunlight.

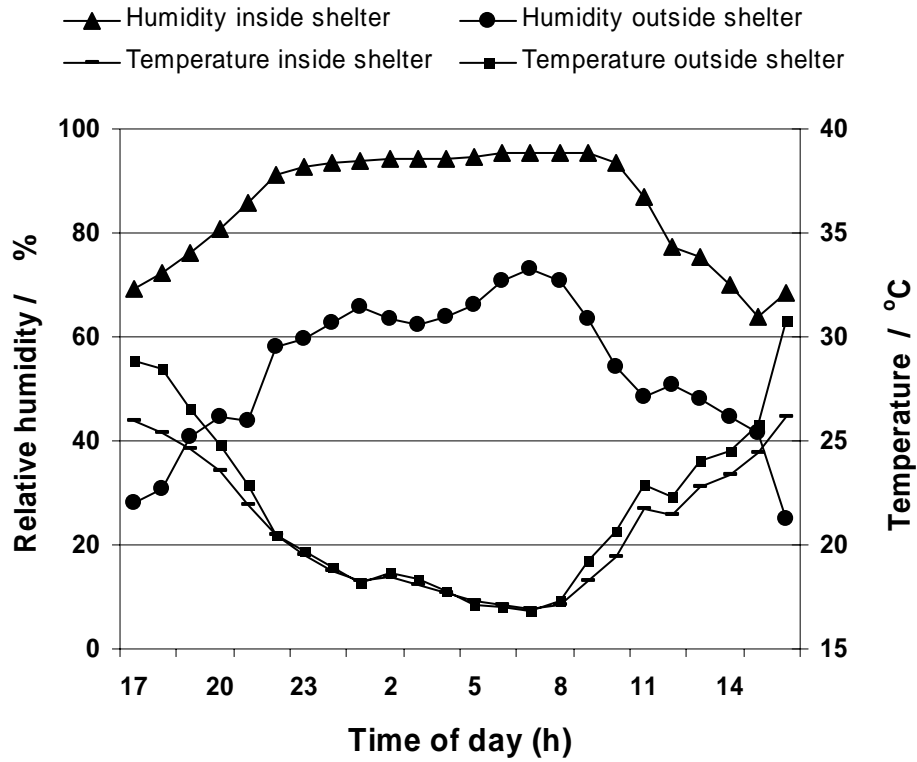


Figure 5: The average density of trichomes on the petiole stem. *H. mantegazzianum* were subjected to either herbivore feeding (with aphids) or to no feeding. Aphids significantly induced the plant defence by producing trichomes in a higher density on the young petiole shoots (ANOVA, $F = 36.8$, $p < 0.001$, with stem diameter as covariate, $n = 36$), but non-significant for the old petioles (t-test, $p = 0.78$, $n = 74$). Error bars indicates + SE.

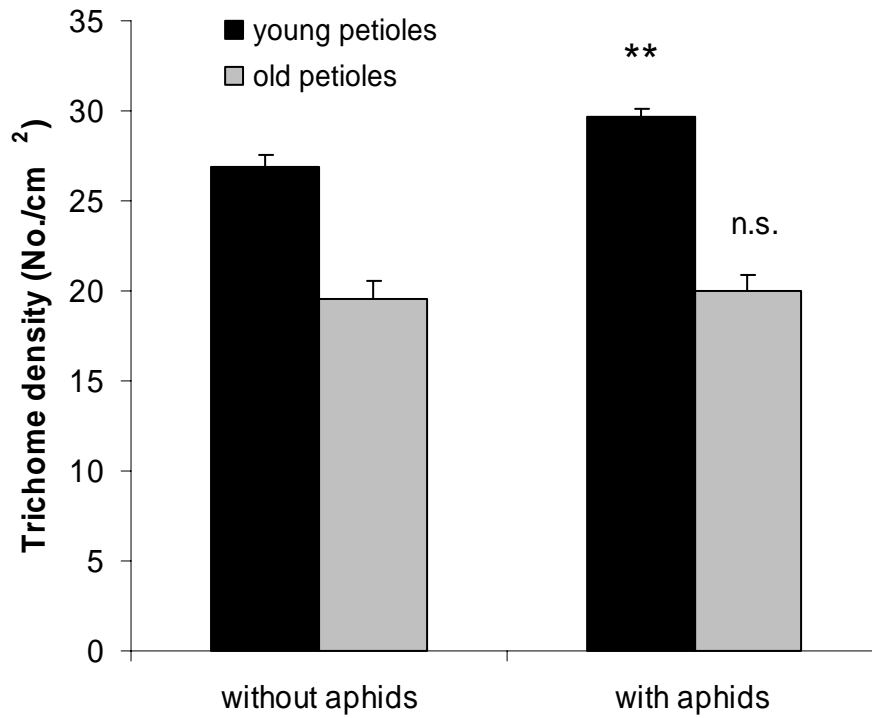
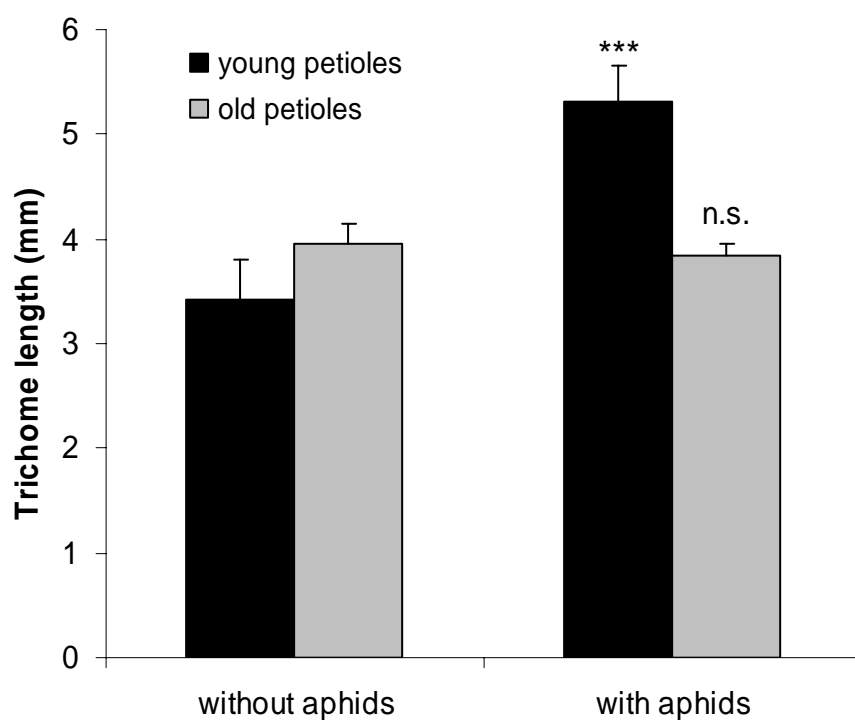


Figure 6: The average length of the trichomes on the petiole stems. *H. mantegazzianum* were subjected to either herbivore feeding (with aphids) or to no feeding. Aphids significantly induced the plant defence by producing trichomes in a higher density on the young petiole shoots (t-test, $p < 0.001$, $n = 36$), but not on the older petioles ($p = 0.61$, $n = 74$). Error bars indicates + SE.



Overall conclusion

Overall conclusion

Until now we did not find any host specific phytophagous insect to employ in classical biocontrol. If we do not find a host specific control agent among those insects we found in Caucasus with still unknown feeding preferences (*Agonopterix caucasella* Zlobin, *Melanagromyza heracleana* Zlobin, *Phytoecia boeberi* Ganglb., and *Nastus fausti* Reitter), or if we do not find additional host specific species of pathogens and herbivores in the native areas, the eradication process will inevitably become very expensive. In fig. 1 (general introduction) we saw *H. mantegazzianum* already covering the majority of the grids within the distribution zone. The short term solution (small short time cost) at in the present stage, is to recognise the weed as a herb that will stay, hoping for a break in the exponential growth (fig. 2, general introduction) and just remove the plant from the localities where it disturbs the most (playgrounds, recreational areas etc.). The alternative long term solution (high short time cost) is to develop an integrated strategy, for all the affected and potentially affected countries (in a common forum such as the EU) and eradicate the weed at the same time. If it results in a sufficiently high eradication, the long term economical cost might be lower. Various methods for control such as root cutting, sheep and cattle grazing, mowing, applying herbicides, etc. have been used, but most of these methods are not applicable for all habitats. One can for example not apply herbicides in an aquatic environment, without severe consequences and it is therefore necessary to combine several methods to be able to reach all locations.

The high rate of 77 % unsuccessful control of weeds in Niedersachsen (table 2 in general introduction), stresses the importance for a more rational strategy, before trying to control invasive weeds. Despite the high failure rate, 86 % of the local authorities believe that future control actions should be attempted, (table 2) and only 14 % voted for a general laissez-faire strategy (Schepker and Kowarik 2001). The Giant alien EU-project was essentially created, in order to find efficient methods for controlling giant hogweed and to avoid unsuccessful control attempts (table 2). Even if control is not yet in sight, the integrated strategy that was developed is a good template for initial investigations of future invasive weeds.

Overall conclusion

We have realized that it is not straight forward to differentiate between plant-damage and plant resistance. This is important for future impact studies and emphasize that herbivore impact should be correlated with either plant growth and certain herbivore load or corrected for the degree of induced plant defence (mechanical, chemical or indirect, Part III) before any damage impact can be quantified.

The results from part I indicate that removing ant colonies from areas invaded with giant hogweed, could lead to a reduced plant growth. Such a solution might however be more difficult than removing the plant itself and the impact is probably not sufficient to control the plant. The observed plant-aphid-ant interactions are nevertheless of great scientific interest. Like most mutualisms the symbiotic ant-plant mutualism probably began as parasitism (Thompson 1982). To understand why ants seem to be predisposed to form these mutualistic relationships and which mechanisms are responsible for coevolved associations, will bring important knowledge about complex systems where mutualistic relations involves 3-4 partners.

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Part I

Steen O. Hansen, Jan Hattendorf and Wolfgang Nentwig: Three partner mutualistic relationship beneficial to all partners: ant-made soil shelters house aphids on giant hogweed.

Part II

Steen Ole Hansen, Jan Hattendorf, Ruediger Wittenberg, Sergey Ya. Reznik, Charlotte Nielsen, Hans Peter Ravn, Wolfgang Nentwig: Phytophagous insect fauna of the Giant Hogweed *Heracleum mantegazzianum* in invaded areas of Europe and in its native area of the Caucasus.

Part III

Steen O. Hansen, Jan Hattendorf and Wolfgang Nentwig: Two plant responses induced by aphids on the invasive Giant Hogweed *Heracleum mantegazzianum*.

Appendix

To determine the proportion of soil shelters on 3-year-old plants the frequency of plants with shelters and the shelters sizes was investigated. Different investigations were performed 2002 and 2003, during two years in four localities in Russian Caucasus on 166 *H. mantegazzianum* plants, from all our visited localities. The species of aphids and ants occurring and their abundances was determined and compared with the constructions of soil shelters. The localities were: Laba river camp N 43° 40' 44'' E 040° 49' 15'', Observatory N 43° 39' 22.5'' E 041° 24' 58'', Mostovskoy N 44° 40' 36'' E 040° 50' 12'' and the previously described locality Pregradnaja. Observations were also made in Mariánské Lázně, Czech Republic N 50° 04' 44'' E 012° 35' 44'' and a locality in the university garden, Bern, Switzerland N 46° 57' 07'' E 007° 25' 41''.

The abundance of soil shelters showed big seasonal and yearly changes. Seventy randomly selected three-year-old *H. mantegazzianum* plants, examined for herbivores from three localities June 2002 in the Caucasus, showed that 37 plants (= 53 %) had aphids inside the leaf envelope (*D. lauberti* or *A. subterranea*). During our investigations 2002-2003 to three localities in Russian Caucasus, *L. niger* constructed 57 % (n = 166) of the observed shelters, *M. rubra* and *M. ruginodis* constructed 39 % of the shelters and 4 % had a uncertain origin.

Ants are known to construct various kinds of structures around food resources and roadways (Anderson and McShea 2005). For example are ants like *Pheidole* spp. (Way 1963) and *Formica altipetens* Wheeler (Cushman and Whitham 1989) building shelters above attended Homoptera. We found ant larvae and pupae under only one soil-shelter, out of more than 200 investigated plants, with soil-shelters constructed by ants. Under all other plants there were observed aphids.

We also observed soil-shelters built by ants above aphids on other plants too. Soil shelters were also constructed by *L. niger*, around aphids *Anuraphis farfarae* Koch feeding externally on the stem of *Petasites hybridus* L. (Asteraceae) in Pregradnaja Caucasus. *M. rubra* build shelters around aphid colonies feeding on *Knautia arvensis* (Asteraceae) and *Macrosciadium alatum* Bieb. (Apiaceae) in the Caucasus.

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Curriculum vitae

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