



Mutualistic relationship beneficial for aphids and ants on giant hogweed (*Heracleum mantegazzianum*)

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Abstract. Giant hogweed (*Heracleum mantegazzianum*), a weed originating from the Caucasus and invasive in Western Europe, is frequently observed with myrmecophilic aphids, ants, and non-myrmecophilic aphids. The number of individuals of the two non-myrmecophilic, leaf-sucking aphid species, *Paramyzus heraclei* and *Cavariella theobaldi*, are negatively correlated with the growth of giant hogweed in its native habitat. A cavity at the stem basis of giant hogweed, the domatium, lodges colonies of the obligate myrmecophilic, stem-sucking aphid *Anuraphis subterranea*. We found a positive correlation between relative plant growth, ant activity, and the number of myrmecophilic aphids. Because of the domatium size, *A. subterranea* populations are limited in growth and consequently the damage they inflict is limited. In contrast to the few other systems where three-partner mutualistic relationships are described, these partners appear to be more adapted to each other. This is the first report of an ant domatium from the temperate zone, and it is moreover the first experimental result presenting a system, from which a secondary domatium is able to evolve, because it is involving initial relations with aphids.

Introduction

Many tropical plant species house scale insects (Homoptera: Coccoidea) and treehoppers (Homoptera: Membracidae), which attract ants with their honeydew. Some ants enter a trophobiosis with homopterans, which provide the ants with food indirectly via the plant (Hölldobler and Wilson 1990, Itino et al. 2001) and honeydew is known to provide a large percentage of the energy budget for some ant species (Horstmann 1974, 1982, Skinner 1980). Ants protect plants from certain herbivores (Buckley 1987, Vasconcelos 1993, Offenberg, et al. 2004), and homopterans from predators or fungal attacks (Buckley 1987, Hölldobler and Wilson 1990, Bach 1991). Ant tended aphids and soft scales remove plant sap and may damage plant tissues or inject toxins and attract pathogens (Bach 1991, Gullan 1997). In contrast, plants bearing ant-homopteran-associations may experience reduced damage from other herbivores (Bach 1991, Vrieling et al. 1991), as plants with extrafloral nectaries sometimes do (Carroll and Janzen 1973, Koptur 1984, Thomson 1994), and these strategies may evolve together at the same time (Offenberg 2000). For example, the ant *Lasius niger* L.

has a strong positive effect on the growth and developmental rate of the aphid *Aphis fabae* Scopoli by quickly repelling adult coccinellids from aphid aggregations by carrying off small coccinellid larvae, but sometimes also regulating the aphid population (El-Ziady and Kennedy 1956, Offenberg 2001).

Ants obligatorily occurring inside living plants have evolved in five out of 12 ant subfamilies in approximately 30 genera (Davidson and McKey 1993). Some ant families are perhaps predisposed to evolve symbiotic associations with homopterans or plants (Davidson and McKey 1993). The hollow space or crevice inside the plant lodging beneficial insects is called a domatium (e.g., Fiala and Maschwitz 1992, Davidson and McKey 1993, Agrawal et al. 2000). A primary domatium occurs when ants invade a weak plant structure (e.g., hollow stems) that instantly provides protection. A secondary ant domatium is an inflated or modified structure that specifically serves to shelter certain ant species (Jolivet 1996).

Benson (1985) found it difficult to see how a myrmeco-domatium in the Amazon, which is too small to shelter ants, could evolve initially. Benson proposed that

they might have evolved from the homopterans feeding sites. The homopterans then become zoological devices used by the plant to maintain the ants near the plant. Another hypothesis explaining how mutualistic associations can evolve between ant and aphids, the plant permissive hypothesis, suggests that also the host-plant quality determines the attractiveness of aphids for the ants (Stadler and Dixon 2005). For such a symbiotic balance to be stable there must also have been a selection for a system where the homopteran populations cannot grow too big and therefore do not kill the plant, and a system where the plant does not defend itself so vigorously that it starves the ant colony (Janzen 1979). Domatia may also provide protection to the inhabitants from inclement weather conditions, from predators and parasitoids, and they may reduce the incidence of diseases (Way 1954, Gullan 1997, Agrawal et al. 2000). Many tropical ants protect their colonies and their honeydew sources by building plant-fibre shelters, thus extending their caring effort into the rainy periods (Way 1963, Benson 1985).

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 (Hölldobler and Wilson 1990, Nakamura et al. 1992, Davidson and McKey 1993, Itino et al. 2001, Jolivet 1996) probably because it is impossible to reside in a plant 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. The good competitive ability and high seed production make it an aggressive invasive species, especially in habitats where 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 (Pysek and Pysek 1995).

The three most common aphids on *H. mantegazzianum* have different feeding places (niches): The non-myrmecophilic aphids *Cavariella theobaldi* Gillette & Bragg and *Paramyzus heraclei* Börner feed on the leaves or umbels sometimes in large densities. *A. subterranea* Walker is myrmecophilic and feeds only inside the leaf envelopes (Heie 1992). The apterous viviparous *A. subterranea* have a very long rostrum ($0.7 \times$ body length) that allows penetration of the thick epidermis of the stem base of large *H. mantegazzianum* plants. The leaf-feeding *C. theobaldi* and *P. heraclei* have a rostrum half this size. Aphids inside a domatium of *H. mantegazzianum* induce the ant *Lasius niger* to build a soil shelter above the

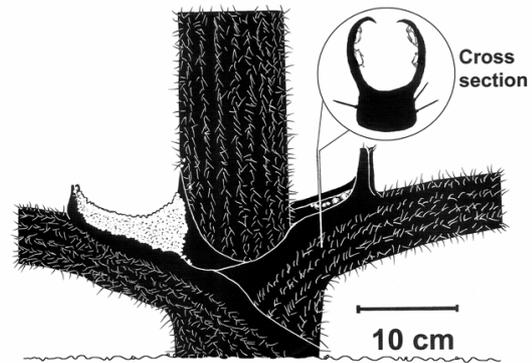


Figure 1. The domatium (leaf envelope) is a hollow cavity at the stem base of a 3-year old *Heracleum mantegazzianum* plant, sheltering colonies of aphids (*Anuraphis subterranea*) and ants (*Lasius niger*). The domatium is curved inwards (see cross section) and ants only construct soil shelters on top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

aphids. Individual aphids are normally located quickly by the ants and seldom found alone (Hansen 2005), probably because of the relatively high ant densities on the ground. Ants are frequently seen foraging on the leaves and stems of giant hogweed.

H. mantegazzianum possesses a domatium made by the petiole envelope at the stem base, regularly sheltering the exclusively myrmecophilic aphid *A. subterranea* and a number of tending ants. These envelopes persist until the plant dies, they are curved inwards to form a cavity partly covered from above, and the ants coat this cavity with soil particles if *A. subterranea* is present. *A. subterranea* is normally found in this part of the envelope (Fig. 1). When the *H. mantegazzianum* plants are approximately one year old the domatia start to open up. In the flower setting stage, the envelopes can enclose a volume up to 50 cm^3 .

In this study we focus on the net outcomes of the relationship between four interacting organisms. How is plant growth connected with ant activity, and the population growth of myrmecophilic and non-myrmecophilic aphids? The following four hypotheses (two direct and two indirect) were tested in our field experiment with a multifactorial design: 1) Each of the aphid species has a direct negative impact on the plant growth. Manual aphid removal and removal with insecticide will therefore lead to increased plant growth. 2) Nutrients directly benefit both aphids and plants. Nutrients 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, both plant growth and aphid numbers will increase when fertilizer is added. 3) Ants are positively correlated

with the number of myrmecophilic aphids (Heie 1992) and ants have an indirect positive impact on the plant because they are known to repel other herbivores. Repelling ants with an insecticide will therefore reduce plant growth. 4) Ant-made soil-shelters are beneficial for both myrmecophilic aphids and ants, and because of hypothesis 3 shelters will lead to an overall positive impact on the plant growth. Artificial clay shelters have a similar effect on plant growth and aphid numbers as ant-made soil shelters do.

Material and methods

The main experiment was conducted in 2003 in Pregradnaja at the northern slope of the Caucasus in Russia (N 43° 54' 26.9'', E 041° 17' 03.2''). The main study locality is a 2 ha clearing partly surrounded by forest, the Teplaya River and a road. The area is an abandoned agricultural field where plants such as *Petasites hybridus* L., *Chaerophyllum aureum* L., *Alcea rugosa* Alef., and *Dactylis glomerata* L. were abundant. Previous experiments have shown that placing aphids inside an empty domatium on giant hogweed induces the ants (*L. niger*) to build soil-shelters around the aphid colonies (Hansen 2005). For the experiment, we randomly selected *H. mantegazzianum* plants with ant made soil shelters and therefore already had both ants and myrmecophilic aphids. The experiment started at the end of June, ended one month later, and all measurements were conducted before and after the treatments. For each plant, the individuals of non-myrmecophilic aphids, *C. theobaldi* and *P. heraclei*, feeding underneath three leaves and *A. subterranea* feeding inside the leaf envelope were counted. The average increase in *C. theobaldi* and *P. heraclei* feeding on the leaf underside of the smallest, the medium sized, and the biggest leaf, respectively were calculated to obtain an estimate of the magnitude of their attack on the whole plant. Individuals of *A. subterranea* in the leaf envelopes were counted and the ant activity was measured as the number of ants running in a 10 cm radius on and around the stem base during one minute. All data were collected daily between 9 a.m. and 5 p.m.

The experiment had a 3 × 4 factorial design (Table 1), where the three factors (treatments): 1) insecticide added, 2) manual removal of aphids, *A. subterranea*, inside the leaf envelope, and 3) control were combined equally with the following four factors (treatments): a) soil shelter removal, b) constructing an artificial shelter on top of the original shelter, c) adding fertilizer, d) control. The treatments were: 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

Table 1. The 3 × 4 factorial design and the number of replicates. 12 different treatments were applied to the leaf envelopes of 96 giant hogweed plants.

	Control	Insecticide application	Manual removal of aphids
Control	8	8	8
Soil shelter removal	8	8	8
Artificial soil shelter created	8	8	8
Fertilizer application	8	8	8

were carefully removed with forceps. a) Soil shelters were removed manually with a spoon. b) Clay shelters are formed and placed on top of the leaf envelopes, with small openings to allow ants tending the aphids and admitting airflow similar to that of the initial soil shelters. 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.

To compare the growth of the plants, the following plant variables were measured: 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 nine umbellets in the primary umbel (three umbellets from the periphery, three from the centre, and three 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 is transformed to 0 and SD becomes 1, Zar 1996), the seven standardized plant variables were aggregated into a single variable called “relative plant growth”, by calculating the average of the variables. The z-standardization procedure causes each variable to have similar size and standard deviation and consequently each variable has a comparable impact on the “relative plant growth” parameter. To make interpretation easier, the value of 1 was added to the relative plant growth (in Fig. 2a and 3a). By combining all seven plant-variables into one, it is possible to give a more precise estimate of the total relative increase in plant size, even when different plant organs sometimes grow differently. The volume of the leaf envelope was measured by pouring defined quantities of water into it and the soil shelter surface area was estimated with a tape measure.

All 3 × 4 combinations were applied randomly to 96 selected three year old plants, because only plants of this age had an envelope open enough for the ants and aphids to enter. Each treatment factor combination had 8 replicates per cell (Table 1). In the beginning of the experiment, there was no significant difference in aphid num-

bers, ant activity, shelter sizes, and plant sizes between the different treatments. Plant growth, aphid numbers in domatia, and 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 (Howell and Games 1974).

Results

The insecticidal and manual removal of aphids significantly reduced the numbers of *A. subterranea* as ex-

pected (Fig. 2d), and both treatments simultaneously reduced the plant growth (Fig. 2a, 2b) (MANOVA and Tukey test). When fertilizer was added, the plants grew significantly more (Fig. 3a). At the end of the experiment in July the ant-made soil shelters had a mean surface area of $18 \pm 23 \text{ cm}^2$. By removing the soil-shelter the number of leaf 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 logarithmically transformed ant activity when performing the MANOVA.

The numbers of *A. subterranea* had a positive impact on the plant growth and there was a significant correlation between plant growth and aphid numbers (Table 2). The numbers of *A. subterranea* were significantly correlated with the ant activity (Table 2). In contrast, there is a nega-

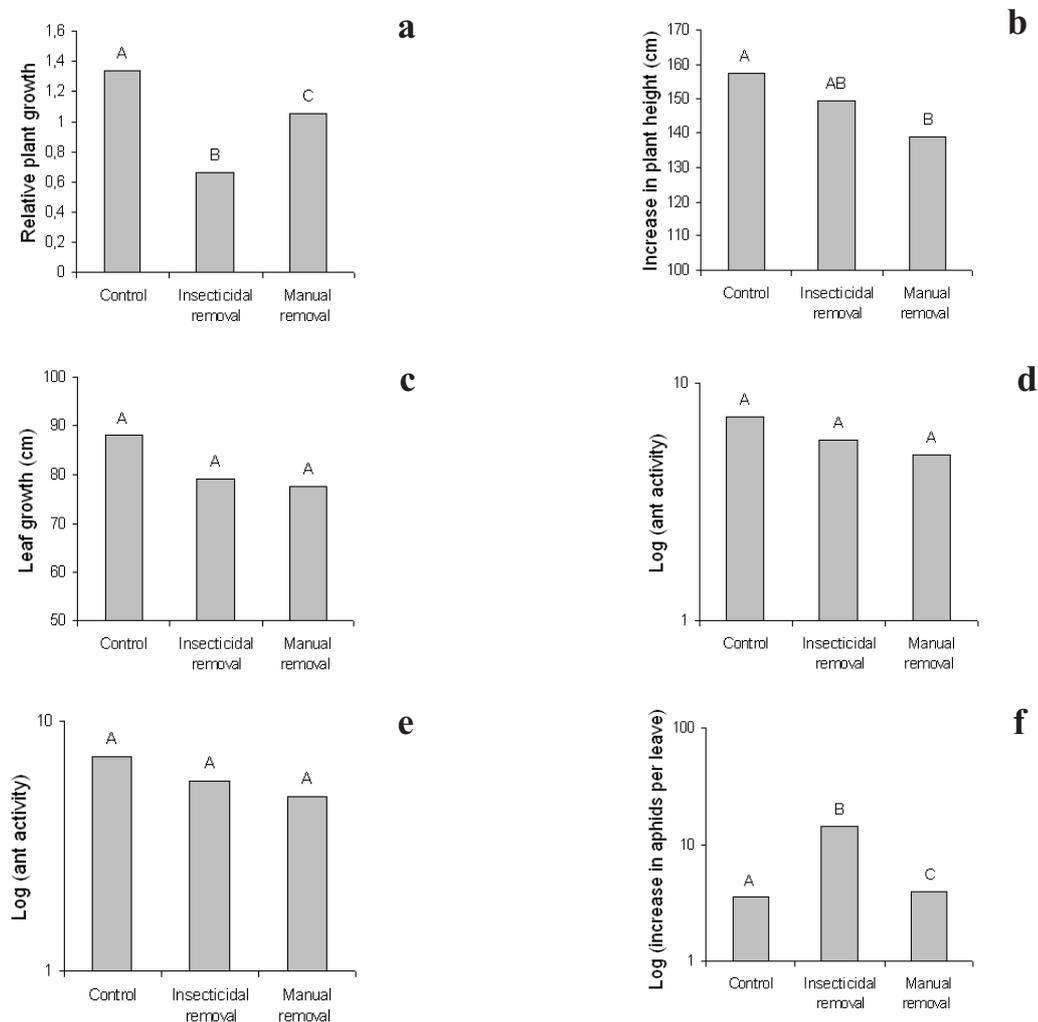


Figure 2. Impact of insecticidal and manual removal of aphids feeding on leaf envelopes of *H. mantegazzianum* on: (a) relative plant growth, (b) increase in plant height (cm), (c) leaf growth (cm), (d) final number of aphids in domatia, (e) ant activity (min^{-1}), (f) increase in aphid numbers per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

Table 2. 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 * $p < 0.0083$, marginally 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)

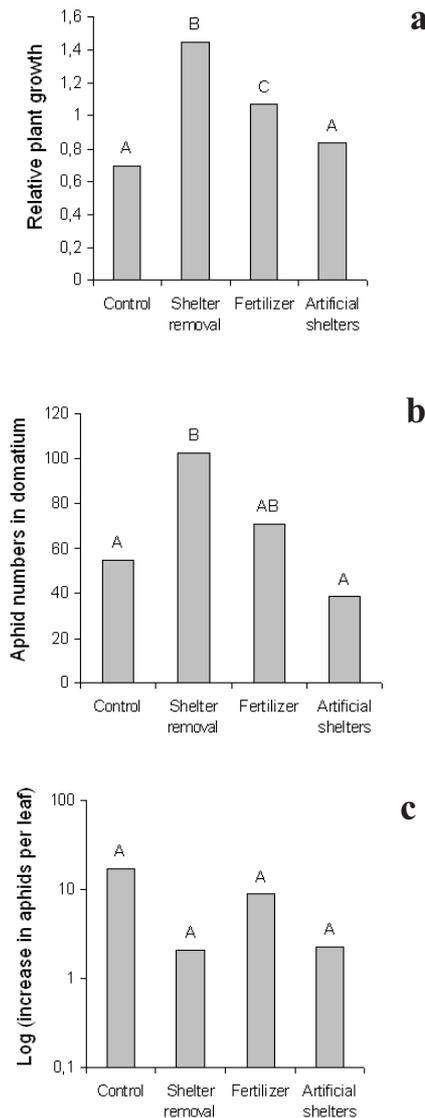


Figure 3. Impact of soil shelter removal, application of NPK-fertilizer and building artificial clay shelters on: (a) relative growth, (b) final number of aphids in domatia, (c) log (increase in aphid numbers per leaf). Different capital letters indicate significance after a MANOVA and a post hoc Tukey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

tive correlation between the number of leaf aphids (*P. heraclei* and *C. theobaldi*), the increase in plant height (cm), and the ant activity (Table 2). An overall average of 82 ± 79 aphids (SD) were feeding on the leaves. The MANOVA with the following post hoc Tukey test, however, showed that no treatment had a significant impact on the number of aphids on the leaves (Fig. 2f, 3c). There was also no significant effect of the treatments on the ant activity (Fig. 2e). The constructions of the artificial shelters did not have impact on this system (Fig. 3a, 3b, 3c).

Discussion

Plant-aphid-ant interactions

Hypothesis 1: The negative correlation between plant growth and the increase in number of aphids on the leaves (*P. heraclei* and *C. theobaldi*) indicates that these species damage the plant (Table 2). The average of 82 aphids on the leaves (which we analyzed carefully) probably cannot have the observed impact alone, but is representing the size of the herbivore load. The number of leaf aphids is closely correlated with the much higher number of aphids in the umbels (which we did not analyze in detail) and both groups together cause the observed impact. A few aphids still existed after insecticide application or manual removal (Fig. 2d). Obviously some survived the treatment or some migrated to the treated plants. In spring or early summer, alate individuals of *A. subterranea* migrate from the primary (*Pyrus communis* L.) to the secondary host plant (giant hogweed). Their offspring 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 their migration (Weber 1968). 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. We can conclude that myrmecophilic aphids exert two opposing 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 2) indicates that the positive effect by attracting ants is stronger than the negative effect by sucking aphids. We can therefore accept our first hypothesis for non-myrmecophilic aphids but not for myrmecophilic aphids, since only non-myrmecophilic aphids are negatively correlated with the plant growth. Manual removal of the myrmecophilic aphids also leads to increased plant growth and demonstrates that *A. subterranea* is beneficial for plant growth and increase in height (Fig. 2a, 2b). The negative correlation between the myrmecophilic and the non myrmecophilic aphids is not significant (Table 1). It shows that ants concentrate on the myrmecophilic aphids, and may benefit the plant performance when the aphids in the umbels or other herbivores (not measured) are deterred by the ants.

Hypothesis 2: Aphids probably would prefer fast growing plants, as their sap contains higher amounts of nitrogen and sugars. This influences quality and quantity of the honeydew-rewards to the ants produced by the aphids and hence the degree of protection (number of ants) that the ants provide to them in return (Messina 1981, Baylis and Pierce 1991, Stadler and Dixon 2005). 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 since plant growth increased (Fig. 3a) and therefore we assume that nutrients did not limit aphid population growth. Hence, Hypothesis 2 is accepted concerning the plant growth but rejected for both types of aphids.

Hypothesis 3: Our results (Table 2) support the third hypothesis, which stated that ants are positively correlated with plant growth because ants protect the plant from certain herbivores. Among the diverse insect fauna associated with *H. mantegazzianum* there are more than 13 aphid species, and eight of these are specialised on Apiaceae (Hansen et al. 2006). Half of these aphid species are not tended by ants and are potentially prevented from feeding by the activity of ants. When, for example, *P. heraclei* is disturbed slightly, it will drop to the ground while emitting an alarm pheromone that makes all individuals in the vicinity drop to the ground as well. This phenomenon explains the negative correlation with ant activity for this species. We rarely saw *L. niger* actually carrying aphids away, but plants are also protected by ant pheromones placed on the plant. *L. niger* produces various pheromones, for example a trail pheromone from its rectal gland (Aron et al. 1993). This has recently been shown to repel herbivores from settling down (Offenberg et al.

2004) but further studies are needed to prove whether these results can be generalized. A plant will benefit from its smell of ants, if the herbivores avoid this smell and if the plant attracts ants it can be considered a plant defence system. The other aphid *C. theobaldi* does not exhibit the same drop down behaviour, but it is also negatively correlated with ant activity (Table 2) and this is probably also a result of disturbance or repulsion by ants. Commonly encountered oligophagous herbivores (Hansen et al. 2006) may also be repelled and consequently increase plant growth.

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 the form of excrements (Jolivet 1996, Benson 1985), and they hold temperature and humidity at moderate levels. *Lasius niger* and other ant species influence the microbial activity and C mineralization rate of the mounds (Dauber and Wolters 2000), thus, ant nests are often surrounded by species rich vegetation (Hölldobler and Wilson 1990). 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. Removing soil shelters has a high impact on the plant growth (Fig. 3a), and the numbers of ants and aphids in the domatia are highly correlated. The aphid numbers are not diminishing significantly when artificial soil-shelters are created, but increasing significantly when the ant-made shelter is removed (Fig. 3b). Another explanation for the correlation between ant activity and plant growth is the combined effect of both increased bioturbation and changed fecundity/mortality/repulsion for the herbivores due to the activity of ants.

Hypothesis 4: Ants frequently build protective covers over aphid and coccid aggregations (Way 1954, Gullan 1997). In our system the important valuable effect for aphids and ants, is that the ant made soil shelter prevents the domatium from getting flooded during rain. In other studies artificial domatia also increased the numbers of predaceous bugs to the benefit of the plant (Grostal and O'Dowd 1994, Agrawal et al. 2000), whereas herbivores such as aphids, spider mites and whiteflies decreased in number. We can partly accept hypothesis 4 because artificial clay shelters have a similar effect on plant growth and aphid numbers as ant-made soil shelters do.

The ant-made and the artificial shelter however appears to limit the plant growth and the aphid numbers (Fig. 3a, 3b), and the fourth hypothesis is therefore partly

rejected, because the soil shelters are not beneficial to *A. subterranea*. When the soil shelters are removed it leads against our expectations to an increased plant growth and aphid increment inside the domatia (Fig. 3a, 3b). The reason why a soil shelter seems to hinder the increase in the aphid population is difficult to explain. One explanation is that soil disturbances induce *L. niger* to recruit an increased worker force which leads to increased ant foraging in the period immediately following (we were not present during this inter-experimental period), and that the ants tending activities might have been beneficial for the plant and 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 (Morales 2000), and the ants also profit from the homopterans they tend (Hölldobler and Wilson 1990). It would seem impossible for an aphid colony to live inside a domatium without tending ants because pathogens quickly would spread when honeydew accumulates. Ants remove the honeydew and produce an antibiotic substance from their metapleural glands (Gullan 1997).

It is more difficult to accept that a plant is also benefiting from a tritrophic interaction that involves herbivores. The pioneer study of Messina (1981) probably demonstrated this for the first time and in recent years evidence for such interactions is accumulating (see the re-

view of Heil and McKey 2003). Our results with giant hogweed are similar to the results from Messina (1981) 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 aphid *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 (Buckley 1983, O'Dowd and Catchpole 1983, Thompson 1994). 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 benefit even when the third species is not present (see the review of Bronstein and Barbosa 2002). 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?

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*

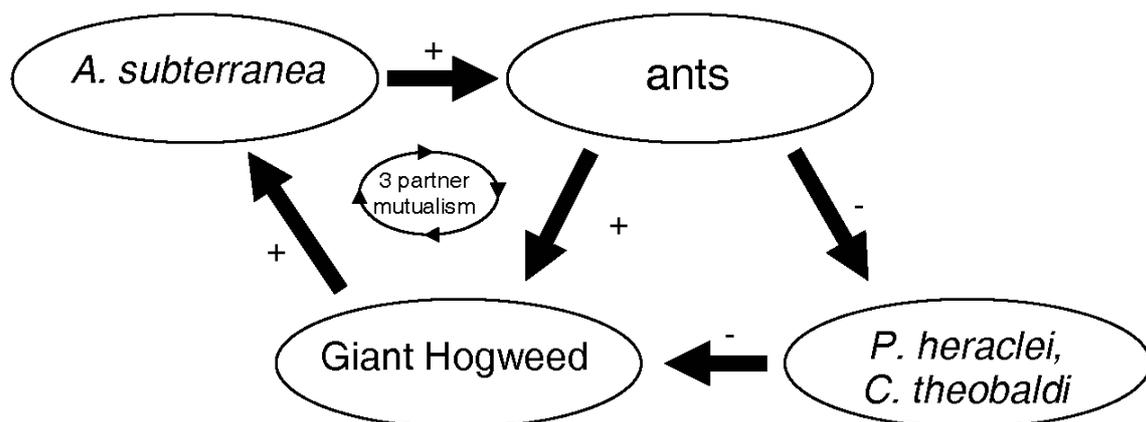


Figure 4. Net growth interactions between four different partners: The ants (*Lasius niger*), the myrmecophilic aphid (*Amuraphis subterranea*), the non-myrmecophilic aphids (*Paramyzus heraclei* and *Cavariella theobaldi*), and giant hogweed (*Heracleum mantegazzianum*).

(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* is among the most widespread and non-specialised ant species and tends numerous aphid species in various habitats. Giant hogweed often grows in habitats where *L. niger* normally lives and ants are therefore not limiting the mutualism. *L. niger* is also a good partner for the plant because it is a relatively aggressive ant that provides good protection against herbivores (Vrieling et al. 1991, Davidson and McKey 1993).

It has been postulated that leaf-pouch domatia of ant-plants in the tropics have evolved from domatia that shelter mites (Schnell et al. 1968, Davidson and McKey 1993, Grostal and O'Dowd 1994). The hypothesis by Benson (1985) states that some secondary leaf-pouch domatia might have evolved from small depressions of leaf surfaces sheltering ant-tended homopterans. 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 and consequently limited damage the plant. The envelopes are moreover 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. *L. niger* is also able to regulate its tended myrmecophilic aphids by preying on some, thus preventing too large aphid populations (Offenberg 2001), although we did not observe those incidents.

We see several hints that the domatium of *H. mantegazzianum* is developing into a secondary domatium. The envelope is already inflated and curved inward, it lacks trichomes and is regularly inhabited by aphids and ants which together with the plant appear to be benefiting from the whole relationship. The association to the aphid *A. subterranea* is intimate, and the aphid is rarely observed alone. Furthermore, the giant hogweed system is equivalent to some of the further developed systems in some tropical ant-plants, and therefore supporting Bensons hypothesis (1985) on how the secondary domatium can evolve via interactions with aphids. The leaf envelope of *H. mantegazzianum* appears not to be a simple natural cavity (primary domatia) but rather the product of joint adaptations and co-evolution.

But what is preventing *A. subterranea* colonies from over-exploiting the plant and maintaining the relationship as a mutualism? *A. subterranea* only seldom appears out-

side a domatium or the 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 (Fig. 1) and probably inhibit aphids from sucking there. Domatia are the only aboveground plant organ lacking trichomes and this again indicates the intimacy of this mutualistic relationship. Thus, the number of aphids in a domatium is regulated by the size of the domatium, i.e., by the plant itself.

The evolution of primary domatia can easily be imagined. Here ants invade cavities of plants and provide instant protection to the plant. The evolution of secondary domatia is different and needs more time to evolve (Benson 1985). Our system presents a non-tropical ant-plant system, where many of the necessary requirements exist to enable its development into a secondary domatium, without the use of extrafloral nectaries or food bodies as assumed by Jolivet (1996), but instead involving the leaf envelopes and aphids. If a mutual partnership is beneficial to a plant and a sheltering domatium provides higher fitness for plants, aphids and ants, there will probably be a natural selection towards inflated envelopes that provide even better protection. In the plants present stage, these envelopes are already large and curved inward (Figure 1), but one could 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 future evolution the plant will try to attract ants without attracting the aphids. 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 *Delpyodora macrophylla* Pierre (Sapotaceae) in southern Cameroon, housing the timid ant *Technomyrmex* (Davidson and McKey 1993). This is therefore an example of one of the next steps in the evolution of a secondary domatium. The final steps in the evolution of myrmecodomatia can be seen in more than 25 tropical plant families (Jolivet 1996): e.g., *Dischidia rafflesiana* Wallich (Asclepiadaceae), *Endospermum formicarum* Beccari (Euphorbiaceae) and more than 65 rubiacean species such as *Myrmecodia* spp. Jackson.

A too specialised mutualistic relationship is perhaps not ideal for the opportunistic nature of *H. mantegazzianum* that 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 breakdown has been considered as the main reason as to why domatia do not occur in the temperate regions (Jolivet 1996). To the best of our

knowledge, the *H. mantegazzianum* case is the first description of a secondary domatium structure from the temperate zone.

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