Examensarbete

Effects of interplant defence signalling on production and allocation of biomass

Kristina Grill

Handledare: Velemir Ninkovic

Institutionen för entomologi
SLU, Box 7044, 750 07 Uppsala
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ABSTRACT

Barley plants are known to release volatiles, both when infested by aphids and uninfested. Neighbouring barley plants have in previous studies been seen to induce a defence mechanism against aphids when exposed to these volatiles. In this study investigated whether volatiles from aphid infested barely plants as well as two chemicals, methyl jasmonate and methyl salicylate, affect biomass allocation and growth of exposed plants.

In order to accomplish this, a first experiment was done in which barley cultivars Alva and Kara were infested by aphids. Other, uninfested, Kara plants were exposed to air from the aphid-infested plants. Plant samples were taken at five occasions and growth parameters were calculated. No changes in total dry weight were seen between the treatments and control plants and no changes in biomass allocation appeared.

A second experiment was conducted, in which Kara plants were exposed to volatiles from methyl salicylate and methyl jasmonate. These two chemicals are thought to be involved in volatile communication between plants and have been shown to induce defence against aphids in barley plants. No change in total dry weight or biomass allocation was found after exposure to the chemicals.

The results suggest that the induced defence in barley plants exposed to aphid infested barley plants, methyl salicylate or methyl jasmonate does not correlate with green plant biomass costs.

SAMMANFATTNING

Det är känt att kornplantor sänder ut lättflyktiga signalämnen, både när de är oskadade och när de är angripna av bladlöss. Dessa volatila ämnen kan inducera försvar mot bladlöss i kornplantor som växer i närheten. Det här examensarbetet har syftat till att undersöka om de volatila ämnen som avges hämmar den totala tillväxten samt om de påverkar den mottagande växten att allokera biomassa till någon viss del av växten.

Två experiment har utförts. I det första placerades bladlöss på kornplantor av sorterna Kara och Alva. Luften som omgav de angripna plantorna fördes kontinuerligt över till oangripna kornplator av sorten Kara. Vid fem tillfällen togs plantor ut för mätning och vägning, för att senare kunna göra beräkningar av tillväxtparameterar. Inga skillnader mellan behandlingarna kunde upptäckas, vare sig gällande total tillväxt eller biomassaallokering.
I det andra experimentet exponerades kornplantor av sorten Kara för två kemikalier, metylsalicylat och metyljasmonat, som inducerar försvar mot bladlöss hos kornplantor och som tros ingå i de volatila ämnen som växter sänder ut. Men

inte heller här sågs några förändringar i biomassaallokeringen eller den totala tillväxten.

Slutsatsen från de försök som gjorts är att den resistens mot bladlöss som uppstår då kornplantor utsätts för volatila ämnen från andra, bladlössangripna kornplantor, eller substanserna metyljasmonat och metylsalicylat, inte ger upphov till kostnader i form av minskad tillväxt.

Abbreviations used in this study

Abbrevition	Means
SLA	Specific Leaf Area
SMF	Stem Mass Fraction
LMF	Leaf Mass Fraction
RMF	Root Mass Fraction
S:R	Shoot: Root ratio
TDW	Total Dry Weight
TLW	Total Leaf Weight
TSW	Total Stem weight
TRW	Total Root Weight
LAR	Leaf Area Ratio
RGR	Relative Growth Rate
ULR	Unit Leaf Rate
MeSA	Methyl salicylate
MeJA	Methyl jasmonate

INTRODUCTION

It is known that many plants, crops as well as plants existing in the natural flora, can interact with each other by emitting volatiles (For review Dicke *et al.*, 2000). The volatiles, often released when the plants have been damaged, can induce actions in the receiving plants, for instance resulting in a defence against herbivores (Reddy *et al*., 2004; Arimura *et al.*, 2001; Karban, 2001; Karban *et al.*, 2004; Agrawal, 2000; Pettersson *et al.*, 1999).

In the future, it might be attractive for farmers to buy plant protection agents containing substances that have been found to induce defence mechanisms in crops. However, few studies have been done costs of induced responses for the plant in terms of reduction of growth (Glawe et al., 2003; Baldwin, 1998; Karban, 2001). The net benefits of interaction between plants are dependent upon such costs (Bruin *et al*., 2001). From an agricultural point of view, it is interesting to know the extent of these costs, before applying results.

Volatile Comunication between Plants

Research has shown that volatiles function in cereal defence against aphids (Petterson *et al.* 1996). Plants can under stress emit substances that protect the plants from herbivores. The processes involved are however very complex and variable. There are many differences between species regarding for instance which chemicals are emitted, how the chemicals work and how long it takes to induce a defence. Chemicals, diseases and insects can induce the defence (Pettersson *et al*., 1996; Walling, 2000; Baldwin, 1998). Pettersson *et al.* (1999) have shown differences in inducing and responding ability between varieties of barley.

Tscharntke *et al*. (2001) has shown that black alder trees (*Alnus glutinosa*) attacked by a leaf beetle larvae (*Agelastica alni*) can induce resistance against the same insect in surrounding trees by emitting volatiles. This effect is probably ecologically important, since the leaf beetle prefers trees standing far away from the emitting tree, both for feeding and egg laying. The emission is a specific response since the alder leaves emit a mixture of chemicals that induce defence in neighbouring trees only when attacked by the leaf beetle (Tscharntke *et al*., 2001).

Wild tobacco plants become more resistant to herbivores when exposed to airborne volatiles emitted from damaged sagebrush. Tobacco plants exposed to sagebrush volatiles produced more flowers and seeds than a control group (Karban, 2001). Damaged sagebrush releases a mixture of different volatiles, best known of which is MeJA, a highly biologically active compound (Preston *et al*., 2001).

Plants that are exposed to herbivore attack can release volatiles that differ from the substances they usually emits in both composition and amount (for review see Dicke *et al.*, 2000).

Transmission of the volatile signals

The distance between an emitting plant and a plant receiving volatiles must not be too large, otherwise the communication is difficult to maintain. In the communication between sagebrush (*Artemisia tridentata*) and wild tobacco (*Nicotiana attenuata*), a distance further than 15 cm appears to be too much. The volatiles must also be received at a physiologically active level in the receiving plant. The greater the distance between the interacting plants, the higher the amount of volatiles that must be released in order to induce a response (Preston *et al*., 2001).

The volatiles emitted from a plant do not only have to have the right concentration, but it must also be possible for a receiving plant to detect them among all other volatiles to which it is exposed. The timing of the exudation can perhaps facilitate the detection of specific volatiles (Bruin *et al*., 2001). Also an abrupt change in concentration of a substance to which plants are normally exposed can serve as a signal (Preston *et al*., 2001).

Why would a plant send out warning signals to other plants? Can this benefit the emitting plant?

Theories

There are several theories on this issue, but to my knowledge no evidence for these theories have been published. It is suggested by Augner (1994) that production of volatiles can help the plant by attracting the natural enemies herbivores. The plant will suffer less damage and therefore achieve greater reproductive fitness (Augner, 1994). Tscharntke *et al*. (2001) implies that it is valuable for a plant to emit volatiles, since they in certain cases can reach other parts of the plant faster than internal signals.

It might be positive for the emitting plant that the receiving plant defends itself when exposed to volatiles, assuming they are close relatives. The plants do not need to be close relatives however in order to share information by volatiles. If sending out such signals leads to a high number of natural enemies of herbivores in the area it is beneficial for most plants, even if other species also can take profit from that (Bruin *et al*., 2001).

The benefits of sending out volatiles are probably dependent on the insects that currently exist in the area (Dicke *et al.*, 2000). The net benefit is likely to differ depending on whether there are many herbivores of a specific species in the area or not. Plants also release volatiles when not attacked or damaged (Ninkovic, 2003), which indicates that there are other benefits to sending out volatiles than to attract or repel certain insects. The emission of volatiles could also be a secondary result of maintaining a constant defence against herbivores. If the plant continuously

produces substances that for instance are repellent to insects, the levels within the plant would become toxic to the plant if nothing were emitted to the surroundings.

For the receiving plant it is useful to be alerted of potential threats in order to be able to start a defence mechanism (Karban et al., 2004). The plant can also react to the threat by allocating growth to the parts of the plant where it is most needed (Ninkovic, 2003).

Costs

The cost of metabolising substances for volatile emission is not very different from producing other cellular metabolites. Enzymes, certain substrates, cofactors and nucleic acid are often required in the production of volatiles (Gershenzon, 1994). It could therefore be suggested that plants that emit volatiles have less energy left for ordinary growth than plants not producing volatiles.

Pettersson *et al.* (1996) suggest that a costly defence leads to less biomass. If too much biomass is lost it will significantly reduce the fitness of the plant.

The substances emitted when a plant is attacked by insects can in certain cases attract herbivores that can damage the plant. This despite the fact that plants often are insect infested, and hence of lower quality as food, if the plants emit volatiles that are easy to detect. The volatiles probably also lead to a higher number of enemies of herbivores in the area. But in some cases, low food quality and enemies are preferable to no food at all (Dicke *et al.*, 2000).

Glawe *et al.* (2003) has found that induction of trypsin protease inhibitors correlates with a fitness cost when trypsin protease inhibitors-producing plants grow in competition with plants lacking these characteristics. Trypsin protease inhibitors act as both direct defences against herbivores, as well as an indirect defence while emitted as volatiles when a plant is herbivore-attacked (Glawe *et al.*, 2003).

If the receiving plant is of different species to the emitting plant, it is not always beneficial for the receiving plant to respond to the signal. Perhaps the insect that has infested the emitting plant is a specialist that does not infest the species of the receiving plant. In that case, the receiving plant does not need to defend itself, since it does not risk an insect infestation (Baldwin, 1998; Karban *et al.*, 2004).

Volatile chemicals involved in plant-plant communication

It is not yet clear whether it is always single compounds or a mixtures of substances that are involved in interplant communication (Bruin *et al*., 2001). The constitution of the volatiles emitted from herbivore-attacked plants depends on the herbivore species and its developmental stage, the plant species, genotype, age and

environmental stress (Walling, 2000). In this study, two chemicals have been used: methyl jasmonate (MeJA) and methyl salicylate (MeSA). Other compounds thought to be important in signalling between plants are for instance some terpenes and certain alkenals and alkanals (Zeringue, 1992).

In field studies, Ninkovic *et al.* (2003) found that treatment with semiochemicals (molecules that carry signals from one organism to another) can delay aphid establishment in a barley crop and reduce the aphid invasion by 25-50%. As semiochemicals methyl salicylate, 2-tridecanone, sulcatol and sulcatone were used. When the crop was attacked at a moderate rate, these substances also gave a reduction in maximum aphid numbers (Ninkovic *et al.*, 2003).

Both jasmonic acid and salicylic acid can activate plant defence genes after herbivore or pathogen attack. When exposed to these chemicals, the plants start to produce toxic compounds and defence proteins (Creelman et al., 1997; for review see Li *et al.*, 2002). It is not known how plant growth is affected when above ground plant parts receive the volatile products of jasmonic acid; MeSA, and salicylic acid; MeJA.

Methyl jasmonate

Jasmonic acid and its derivatives are not only involved in processes such as fruit ripening, production of viable pollen, senescence, root growth and stomatal resistance but also in plant resistance to insects and pathogens (Schultz et al., 2004; Creelman et al., 1997; Pieterse et al., 1999). Jasmonic acid is considered to be involved in resistance inducing reactions not depending on salicylic acid (Pieterse et al., 1999).

In *Nicotiana attenuata* the production of toxic nicotine is activated in the plant by jasmonic acid. When roots were treated with jasmonic acid, the plants that experienced an intermediate rate of attack from herbivores were attacked less often and could survive and produce more seed than untreated plants. If the plants were not attacked by herbivores however, plants treated with jasmonic acid produced less seeds than untreated plants. Hence, jasmonic acid induced defence costs, but on attack, the benefits are bigger than the costs. Jasmonic acid seems to induce similarly reactions in the plant as wounding does (Baldwin, 1998). If the plants are only submitted to jasmonic acid or MeJA when neighbouring plants are attacked and release the substance (and the risk for an attack can be considered to be quite high), it is not very likely that the cost for defence in *N. attenuata* would exceed the benefits.

Methyl salicylate

MeSA is believed to be involved in induction of plant defence. Salicylic acid is an important substance in the processes inducing resistance for diseases in plants,

both locally and systemically; some pathways are even dependent on it (Pieterse et al., 1999).

Salicylic acid and MeSA stimulate the expression of defence-response genes and a systemic resistance (Walling, 2000). MeSA is released from the winter host of the aphid *Ropalosiphum padi*: *Prunus padus* in spring, making the aphids migrate to their summer hosts, and has been seen to act as a plant stress signal (Chamberlain *et al*., 2001). Pettersson *et al.* (1994) demonstrated that MeSA decreases the *R. padi* colonization in cereal fields. The effect of the chemical seems to be dynamic, after a certain age the aphids tend not to respond to it (Glinwood *et al.*, 2000). Arimura *et al.* (2001) suggests that MeSA only can act as an airborne signal between plants when occurring at higher concentrations.

Parameters of growth

Different stresses can affect the growth of a plant. For instance, wheat plants had greater root length when they were aphid infested (Riedell *et al.*, 2003). If the growth of the roots of a plant is affected, the ability to take up water and nutrients will be changed. This will play a major role for the development of the plant and its ability to cope with drought and other physical stresses (Hoffman *et al*., 1997). Damage of a plant can to some extent lead to an increased growth, based on studies from Pedigo *et al.*, 1986.

The increase in biomass of a seedling is proportional to the amount of biomass already present, giving an exponential growth curve. In order to describe at what speed the plant is growing at a certain time, the expression “relative growth rate” (RGR) is used. RGR is defined as “the rate of increase in biomass per unit plant mass already present”. In the photosynthesis the plant gain carbon. Respiration in turn consumes carbon. Photosynthesis and respiration are considered to be the main parts affecting RGR. It can be noted though that volatilisation, exudation and damage by herbivores also consume carbon (Porter, 2002).

The use of growth parameters in research on volatiles can be exemplified by the study of Ninkovic (2003). There, it has been shown that barley cultivar Kara exposed to volatiles from barley cultivar Alva allocates growth to the roots, reduces its LMF and increases its SLA. At the same time the total biomass does not change, nor does the RGR. Alva exposed to Kara volatiles however, does not give any significant changes. The higher SLA of the induced Kara plants is suggested to make the plants able to maintain their RGR (Ninkovic, 2003). Some barley cultivars decrease their leaf temperature if exposed to volatiles from certain other barley cultivars. A low leaf temperature correlates with a higher transpiration rate and influx of CO_2, due to an increased photosynthetic activity (Pettersson *et al.*, 1999).

The aphid

Aphids were chosen as insects in this study since they cause great economic loss in Swedish cereal cropping, and have been used in previous studies. In this study the bird cherry-oat aphid, *Rhopalosiphum padi* L., was used for one of the experiments. *R. padi* is a common problem in oat and barley cropping for farmers in Sweden. In wintertime *R. padi* has *Prunus padus* L. as a host, where the aphid eggs survive. In spring, the eggs hatch and the aphids feed on *P. padus* before they move to their summer hosts, grasses and cereals (Chinery, 1988; Hedene *et al.*, 1994). When *R. padi* reach a plant they test different feeding sites, before they find a suitable place where they can suck plant sap. They usually find a position where they can extract phloem liquid (Riedell *et al.*, 2003).

Once the aphid has settled at a suitable feeding site it can remain there for hours or weeks (Walling, 2000). If the feeding site is too crowded or if the food is not of sufficient quality or amount, then the aphids are triggered to give birth to winged aphids. Volatiles emitted from the host plant can also make the aphids start to migrate, according to results from Pettersson *et al.* (1995).

The extent of damage caused by *R. padi* depends on how many aphids infest the plant, growth stage of the plant and the timing of the infestation during the growth season (Hedene *et al.*, 1994). When a crop is hit by an aphid attack, the size and quality of the yield usually decreases. Therefore, insecticides are often used when the aphid invasion is estimated to cause more economic loss than the cost of insecticide treatment (Hammar *et al.*, 1998).

At a low aphid concentration, wind, rain and predators have more influence on the population than volatiles have. At a high aphid concentration factors within the population, like crowding and emission of volatiles, will have larger impact on the aphid infestation (Ninkovic *et al.*, 2003).

Aims of the study

In this study, the aims are to study how biomass allocation and growth pattern changes in a plant exposed to volatiles emitted by other plants. In order to do this, two experiments were done. One experiment aimed to test the reaction of barley plants (*Hordeum vulgare* L.) when exposed to volatiles from other, aphid infested, barley plants. The second experiment aimed to test the effects of chemicals (MeJA and MeSA) on barley plants.

The hypotheses to be tested are:

- Growth will be reduced in a barley plant when exposed to volatiles produced by other barley plants, attacked by aphids.
- Growth will be reduced in a barley plant when exposed to each of the volatile chemical methyl salicylate and methyl jasmonate.

Specifically the study aimed to provide information on the following questions:

1. Will total plant biomass change?
2. Will some part of the plant be benefited or disadvantaged more than other plant parts?
3. Will the relationship between stem-root-leaves be changed?
4. Will the leaf area increase, and in that case, will the leaf mass also increase?
5. How will the length and mass of the roots be affected?
6. Will the relative growth rate be changed?

MATERIAL AND METHODS

Material

Aphids

The aphids used were bird cherry-oat aphids, *Rhopalosiphum padi* L. They were produced in a greenhouse with a day/night regime of 12/12h and a temperature of 18-22ºC, fed on oat and barley.

Plant material

Different barley cultivars have very different qualities when it comes to inducing and responding ability on volatiles. It was therefore important to carefully choose varieties involved in this experiment. Two varieties of spring barley was tested; Kara and Alva, since they had been used in previous studies. Alva was chosen as the inducing cultivar and Kara as the receiving cultivar in this experiment since Alva has shown to be the most inducing variety and Kara the most responding variety (Petterson *et al.*, 1999; Ninkovic, 2003). Kara is also most inert to self-induction; therefore it is possible to use it as a control (Pettersson *et al.*, 1999).

Methods

Treatments

Two sets of experiments were performed. The first set of experiments (experiment one) was done during springtime, and the second set of experiments (experiment two) in late autumn. In both experiments each treatment was replicated five times for each sampling time.

In experiment one, three different treatments were carried out: Kara exposed to Alva infested by aphids (A*K), Kara exposed to Kara infested by aphids (K*K)

and Kara exposed to Kara not infested by aphids (KK). Kara without aphids exposing Kara was used as a control. A schematic picture of experiment one is presented in figure 1.

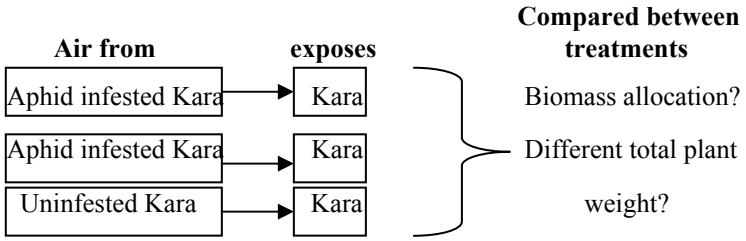

Figure 1. *Schematic picture of experiment one, where barley plants of cultivar Kara were exposed to aphid infested Kara plants, aphid infested barley cultivar Alva plants and uninfested Kara plants. The aim was to see if changes in biomass allocation and total plant weight parameters occurred between treatments.*

In the second experiment, no aphids were involved; instead Kara plants were exposed to MeSA, and MeJA. Two types of control conditions were included; Kara exposed to volatiles from Alva and Kara exposed to air without plant volatiles or chemicals. The last condition was added in case all treatments gave the same result. If Alva provoking Kara did not differ from the control, it would indicate that something was wrong with the environment in the greenhouse, since it has been shown that Alva provoking Kara gives rise to a significant change in growth (Ninkovic, 2003). A schematic picture of experiment two is given in figure 2.

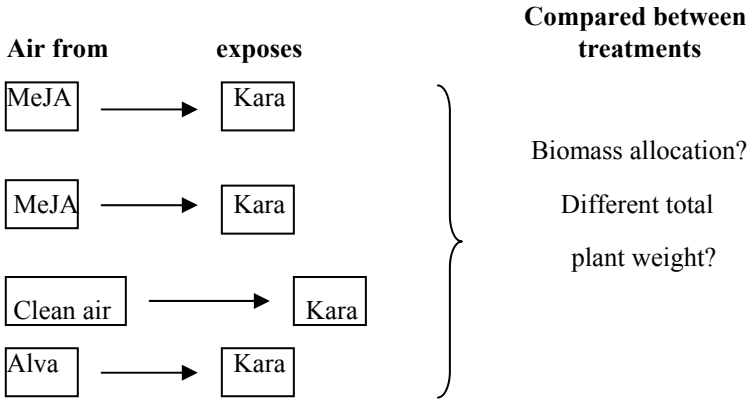

Figure 2. *Schematic picture of experiment two, where barley plants of cultivar Kara were expoed to MeJA, MeSA, clean air and barley plants of cultivar Alva.*

Exposure to volatiles

'Twin-chamber cages' (Pettersson *et al*., 1999) were used in both experiments one and two. A twin-chamber cage consists of two plastic chambers next to each other, separated by a plastic wall (figure 3). The top of the cage is open. During this experiment it was closed with cling film and rubber bands. The irrigation tubes were drawn through small holes in the walls of the chambers to reach sand bags (serving as plant pots) that were placed in the middle of each chamber.

Air was taken into chamber one (inducing chamber) through a hole (7 cm diameter), covered with a thin net that kept the aphids in experiment one from escaping. The air passed through the wall via a similar net-covered hole and into chamber number two (responding chamber). A vacuum tank drove the airflow by sucking the air through a hole at the top of the responding chamber. The air was then vented outside the greenhouse. The airflow was kept at a rate at which in each chamber (10×10×40 cm) was completely replaced every second minute.

Experimental details
Barley seeds were grown for one day at room temperature in Petri dishes on filter paper moistened with distilled water. The germinated seeds were placed in plastic bags (1×0,05 m) filled with clean wet sand. One seed was placed in each bag at 1 cm and 2 cm depth respectively for experiment one and two. The bags had been perforated with needles in order to allow aeration.

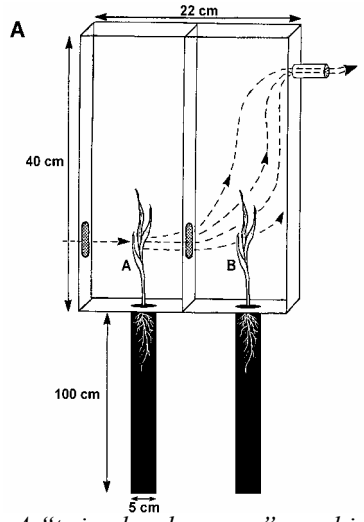

Figure 3. *A "twin-chamber cage", used in both experiments. Under the cage were plastic bags filled with sand for the plant roots. The plant in chamber (B) is exposed to air from chamber (A). In the second experiment chemicals on a filter paper was placed in chamber A instead of a plant.*

Two holes with a diameter of 3 mm in the bottom of each bag ensured that surplus water and nutrients would be able to exit. The bottoms of the bags were placed in plastic draining-pipes, which were rinsed through once or twice a day to avoid algae growth. Separate bags for each plant were used in order to avoid root exudates affecting the communication. Since roots can emit substances that affect other plants, it is important to make sure roots from different plants do not have contact in order to study above ground communication between plants (Bruin *et al*., 2001).

The germinated seeds prepared for the different treatments were placed randomly in two greenhouses, which had specially made benches. The benches had 5×2 cm holes in which the tops of the bags were placed and fixed by staples. Foam plastic plugs were placed between the bags and the bench to seal the holes properly. The plugs prevented light from reaching the roots and air from exiting or entering the chambers. The bags were also protected from light by a black plastic curtain hanging down from the bench, reaching the floor.

A day/night light regime was imposed at 16 h day and 8 h night. Artificial light automatically started as the sunlight went below 200 W/m^2.

Experiment one was started nine days after germination; six aphids per treated plant were placed on stems and leaves.

In experiment two, 10 µl of methyl salicylate (98%) or 10 µl of methyl jasmonate (95%) were added every day, starting on day eight. The chemicals were placed on a filter paper in an open Petri dish, which was placed in the inducing chamber in the twin–chamber-cage. When adding new chemicals, the Petri dishes were closed and taken to a separate room, where the filter paper was replaced with a new one, upon which the new chemicals was placed. The replacement of the filter paper was done in order to ensure no chemicals were left from the day before in the Petri dish, with risk of accumulation of the substances. Then the Petri dish was again closed, brought into the greenhouse and placed open in its original chamber. Thus, a minimum of volatiles escaped outside the cages.

Watering

In experiment one, the sand was kept wet all the time by an automatic drop-irrigation system that watered for three minutes with ten minutes intervals, except for the first two days, when the sand was watered constantly and without nutrition. Otherwise the water always contained a nutrition mixture Wallco 51-10-43 + mikro (containing 20 g NH_4^+, 31 g NO_3^-, 10 g P, 43 g K, 4 g S, 3 g Ca, 4 g Mg, 0,17 g Fe, 0,2 g Mn, 0,1 g B, 0,03 g Zn, 0,015 g Cu, 0,0004 g Mo per litre, free from Cd, Cl and Na, all substances except B chelated) with an electrical conductivity, EC, about 1 mS/cm. pH of the nutrient solution was adjusted to 6,9. In the first experiment the irrigation strategy was changed at day 16 to watering 40 minutes every 15 minute, because of problems with the EC. In experiment number

two, the irrigation water always contained nutrients and the plants were watered 10 minutes every 10-minute. The EC was then kept stable around 1 mS/cm.

No exact figures of water and nutrients added are available, but since all plants received the same amount of water and nutrients, this does not affect the reliability of the results.

Sampling

Samplings were taken randomly at five different occasions, starting twelve and eleven days after germination in experiments one and two respectively. The sampling continued two times a week with about three days between each sampling. The experiment was ended when the last sample was taken, at day 27 in experiment one and at day 24 in experiment two. Many of the plants in the Alva provoking Kara treatment in experiment two did not germinate after placement in the sand, and therefore only four replicates could be measured at time four and no replicates at the last time.

Each sampling consisted of five twin-chamber cages and the plants therein. The irrigation tubes and the cage were taken off. Then the number of aphids was counted in experiment one and they were removed from the plant. The bags containing sand and a barley plant were removed from the bench and draining-pipe. The plants were separated from the sand; the roots were dipped in water twice to take away remaining sand and then carefully dried with kitchen paper.

With a ruler the length of the roots, the shoot and the separate leaves were measured. The plant was divided into roots, leaves and stem, wrapped in aluminium foil and weighed. The leaf area was measured for each leaf with a Li-3100 (Li-cor, Lincoln, Nebraska, USA) leaf area meter. All plant parts were then dried in 70°C for 48 hours, and thereafter weighted again after cooling for one hour.

Calculations and parameters

Biomass fractions, divided into at least leaves, stems and roots, are preferred to using shoot:root ratios. This is because ratios are sensitive to small changes in allocation (Poorter *et al.*, 2000).

Data from the measurements were used for statistics, and calculation of morphological parameters of growth; specific leaf area (SLA), leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), leaf area ratio (LAR) and shoot: root ratio (S:R).

SLA is the total leaf area divided with the total leaf dry weight. LMF is the dry weight of leaves divided with the total dry weight (TDW) of the plant. Similarly,

all fractions are the dry weight of a particular plant part divided with TDW. LAR is the total leaf area divided with TDW. S:R is the above ground dry weight divided with root dry weight.

Physiological parameters of growth were calculated as well; relative growth rate (RGR), unit leaf rate (ULR) and total dry weight (TDW). RGR is an expression for how much biomass the plant has gained over a certain period of time. It can be written as RGR= $(TDW_{T2}–TDW_{T1})/(T2–T1)$, where T1 is time one and T2 is time two. ULR can be explained as the rate of dry weight production in relation to total leaf area over time. ULR = $(TDW_{T2}–TDW_{T1})/(\text{Total leaf area}\times(T2–T1))$.

The parameters are correlated to each other, which can be seen in these formulas:

$RGR=ULR\times LAR$ $LAR=SLA\times LMF$ $RGR=ULR\times SLA\times LMF$
(Hunt, 1990; Hunt, 1978)

If RGR of a treatment is not the same as RGR of the control, it can be due to differences in either ULR or LAR between treatment and control. Changes in LAR can in turn depend on differences in SLA or LMF. Hence RGR is affected by ULR, SLA and LMF.

Statistics was done using Statistica (Wonnacott *et al.*, 1990) and SAS software. All data presented was determined to be normal distributed. Two way ANOVA was used followed by pair-wise comparison of Bonferroni test. Results showing p-values equal or less than 0,05 were considered to be significant.

In both experiments samples of the treatments were taken at five occasions. Comparisons between treatments were made on both average values of a whole treatment and average on one sample occasion. Unless otherwise stated, the results represent average values of a whole treatment session.

RESULTS

Experiment one:
Barley plants exposed to volatiles from aphid infested barley plants

In the first set of experiments three treatments were carried out; Kara exposed to infested Alva (A*K), Kara exposed to infested Kara (K*K) and Kara exposed to Kara (KK). All samplings taken at observation time number four were excluded from statistical analysis since those measurements contained a few errors.

Plants exposed to volatiles

Morphological parameters

None of the morphological parameters (SLA, SMF, LMF, RMF, S:R, TDW, TLW, TSW, TLW+TSW, TRW, LAR and leaf area, leaf length, root length, stem length, and stem + leaf length) show significant changes when barley cultivar Kara plants are exposed to aphid infested barley cultivar Alva plants. All mean values and standard deviations can be seen in appendix, table one. Likewise, there were no significant changes between aphid infested Kara plants compared to Kara exposed to clean air.

There were however some tendencies for effects, evaluated from the complete period of treatment. A*K tend to have a lower LMF than K*K and KK (table 1). K*K tended to have a lower RMF than A*K and KK. KK tend to have shorter root length than A*K and shorter stem than A*K and K*K (table 1).

Physiological parameters

No significant changes of RGR and the physiological parameter ULR were seen between the treatments.

There were however tendencies regarding both physiological parameters. RGR of K*K tend to be lower than RGR of KK. At measurement 3-4 average of RGR of KK is significantly bigger than K*K ($p=0,03$) and A*K ($p=0,0009$), but in the next period (4-5) KK tend to have less RGR than K*K and A*K (figure 4).

In the case of ULR, a total average of K*K tended to have lower ULR than KK. Significant changes were found at measurement 3-4, where KK has greater ULR than K*K ($p=0,03$) and A*K ($p=0,004$) (figure 5). Mean values from experiment two can be seen in table 1.

Tabel 1. *Results from experiment two. P-values, mean and standard deviation. MS= Barley cultivar Kara exposed to methyl salicylate, MJ= Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. T=Treatment, D=Days, T*D=Treatment *Days, d.f=degrees of freedom. Subscripted letters refer to Bonferroni test.*

			Mean ± standard deviation		
Morphological parameters	**d.f**	**ANOVA**	**MS**	**MJ**	**0K**
LMF	2 4 8	pT=n.s. pD=0,002 pT*D=n.s.	$0,43^{a} \pm 0,06$	$0,41^{a} \pm 0,1$	$0,41^{a} \pm 0,07$
RMF	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$0,38^{a} \pm 0,1$	$0,37^{a} \pm 0,1$	$0,39^{a} \pm 0,1$
Root length (cm)	2 4 8	pT=n.s. pD=0,002 pT*D=n.s.	$24^{a} \pm 9,2$	$20,8^{a} \pm 8,7$	$22^{a} \pm 9,6$
Stem length (cm)	2 4 8	pT=n.s. pD=0,001 pT*D=n.s.	$7,30^{a} \pm 1,0$	$7,52^{a} \pm 1,0$	$7,34^{a} \pm 1,3$
Physiological parameters	d.f.	ANOVA	MS	MJ	0K
RGR (g/g day)	2 3 6	pT=n.s. pD=0,0005 pT*D=0,019	$90,0^{a} \pm 63,6$	$97,1^{a} \pm 45,6$	$79,9^{a} \pm 60,9$
ULR (g/m2 day)	2 3 6	pT=n.s. pD=0,000 pT*D=0,003	$2,77^{a} \pm 2,0$	$2,88^{a} \pm 1,40$	$2,62^{a} \pm 2,0$

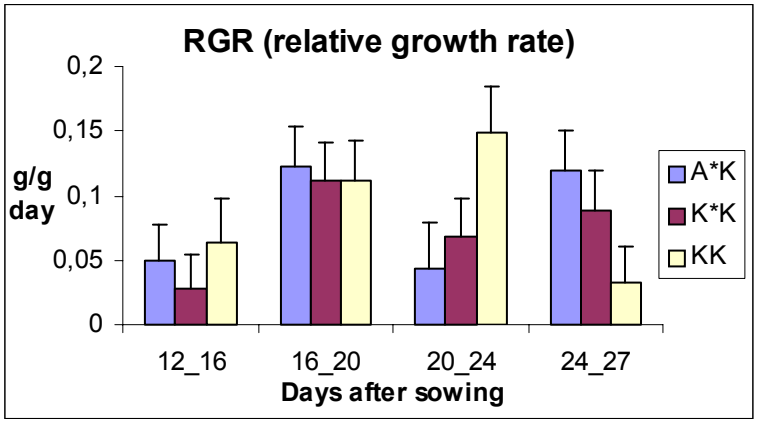

Figure 4. *RGR of experiment one. A*K=Barley cultivar Kara exposed to aphid infested barley cultivar Alva. K*K=Kara exposed to aphid infested Kara. KK=Kara exposed to uninfested Kara. At 20-24 days after sowing KK is significantly bigger than K*K and A*K, but at 24-27 days after sowing KK tend to have les RGR than K*K and A*K.*

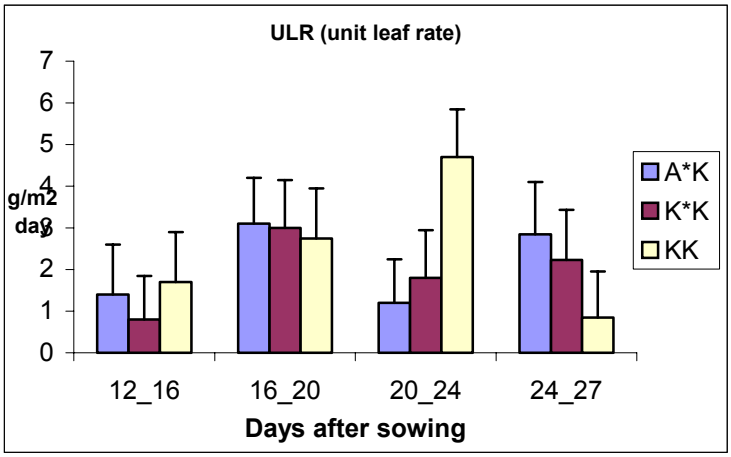

Figure 5. *ULR of experiment one. A*K=Barley cultivar Kara exposed to aphid infested barley cultivar Alva. K*K=Kara exposed to aphid infested Kara. KK=Kara exposed to uninfested Kara. At 20-24 days after sowing KK is significantly greater than A*K and K*K.*

Aphid infested plants

Although the main purpose of this experiment was to investigate how barley plants exposed to volatiles of aphid infested barley plants respond, statistical analysis were also made on the aphid infested plants, since those results were available and could be interesting to explore as well.

Morphological parameters

Kara plants infested by aphids, Alva plants infested by aphids and Kara not infested by aphids did not show any significant changes in the morphological parameters (SLA, SMF, LMF, RMF, S:R, TDW, TLW, TSW, TLW+TSW, TRW, LAR and leaf area, leaf length, root length, stem length, and stem + leaf length). Some tendencies could be found however. Infested Kara tend to have a higher LAR than infested Alva and Kara. Regarding root length infested Alva tend to have greater length than aphid infested Kara and Kara. It was shown that infested Alva tend to have shorter stem than infested Kara and uninfested Kara.

Physiological parameters

RGR and the physiological parameter ULR showed no significant changes between the treatments.

In summary, no significant changes were seen between any of the treatments in experiment one. Tendencies for differences between treatments regarding LMF, RMF, RGR and ULR were however found.

Experiment two:
Barley plants exposed to methyl jasmonate and methyl salicylate

In experiment two, three treatments were carried out; Kara plants exposed to methyl jasmonate (MeJA), Kara plants exposed to methyl salicylate (MeSA) and Kara plants exposed to clean air (control). An extra control treatment, Kara exposed to Alva, was added in toompared witha plants exposed to clean air.

Growth parameters

Morphological parameters

Among the morphological parameters (SLA, SMF, LMF, RMF, S:R, TDW, TLW, TSW, TLW+TSW, TRW, LAR and leaf area, leaf length, root length, stem length, and stem + leaf length) only SLA and TSW showed significant changes between Kara plants exposed to methyl jasmonate (MeJA), Kara plants exposed to methyl salicylate (MeSA) and Kara plants exposed to clean air (table 2).

Tabel 2. *Results from experiment two. P-values, mean and standard deviation. MS= Barley cultivar Kara exposed to methyl salicylate, MJ = Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. T=Treatment, D=Days, T*D=Treatment *Days, d.f.=degrees of freedom. Subscripted letters refer to Bonferroni test.*

Morphological parameters			**Mean ± standard deviation**		
	ANOVA	**d.f.**	**A*K**	**K*K**	**KK**
SLA (m2/kg)	pT=n.s. pD=n.s. pT*D=n.s.	2 3 6	$53,7^{a} \pm 4,5$	$52,3^{a} \pm 9,0$	$54,1^{a} \pm 5,5$
TSW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	2 3 6	$10,9^{a} \pm 7,2$	$9,8^{a} \pm 5,7$	$10,8^{a} \pm 7,9$

SLA of different treatments showed significant changes over time ($p<0,0001$, ANOVA). Bonferroni test made on mean values for each treatment showed that Kara exposed to MeJA gave a significantly higher SLA than Kara exposed to MeSA ($p=0,04$) and Kara exposed to clean air ($p=0,006$). Only measurement number two showed significant changes ($p<0,0001$ ANOVA) when the separate measurements were tested. At measurement two Kara exposed MeJA had a

significantly greater SLA than Kara exposed to MeSA (p=0,0001) and Kara exposed to clean air (p=0,0000) (figure 6).

Kara exposed to MeJA showed significantly higher TSW than Kara exposed MeSA (p= 0,01), Kara exposed to MeJA also tended to have higher TSW than Kara exposed to clean air. All treatments tend to increase their TSW with time (figure 7). Kara plants exposed to MeSA showed no significant changes compared to Kara exposed to clean air. Mean values for TSW and SLA can be seen in table 2.

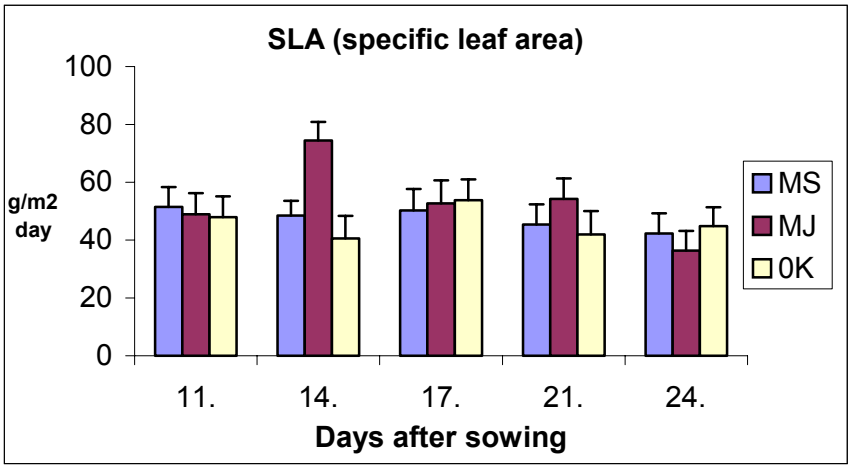

Figure 6. *SLA of experiment two. MS= Barley cultivar Kara exposed to methyl salicylate, MJ = Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. At 14 days after sowing MJ has significantly greater SLA than the two other treatments.*

Physiological parameters

RGR and the physiological parameter ULR showed no significant changes between the treatments (table 2, appendix).

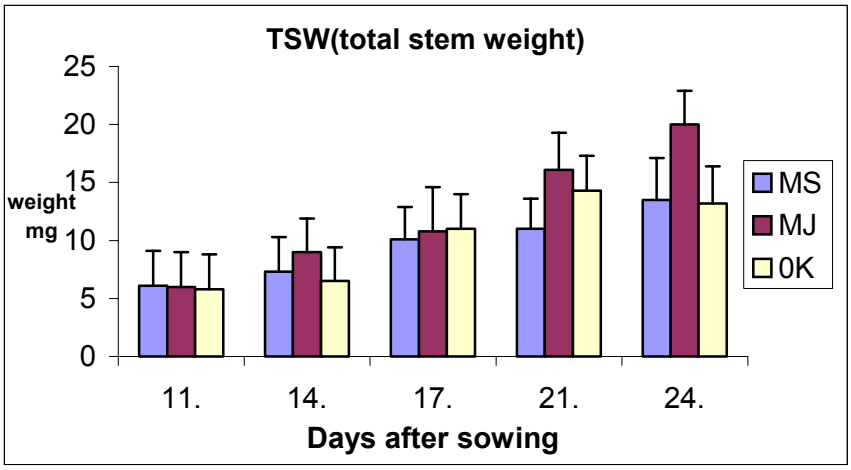

Figure 7. *TSW of experiment two. MS= Barley cultivar Kara exposed to methyl salicylate, MJ = Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. All treatments tend to increase their TSW with time, especially MJ.*

Kara exposed to Alva

Kara exposed to Alva was not included in the statistics since many plants died, and at the last measurement no samples were left to analyse. The treatment was added to the experiment in order to be compared with the control, Kara exposed to clean air. Comparing the results in this study with those in Ninkovic (2003) was intended to evaluate the conditions in the greenhouse. If the result of both studies agreed the conditions were thought to be satisfactory. LSD test in this study showed no significant changes between Kara exposed to clean air and Kara exposed to Alva in any parameters.

In summary, Kara exposed to MeJA showed significantly higher SLA than Kara exposed to MeSA and Kara exposed to clean air. Kara exposed to MeJA also showed significantly higher TSW than Kara exposed to MeSA.

DISCUSSION

Experiment one:
Barley plants exposed to volatiles from aphid infested barley plants

No changes in biomass

Volatiles from barley plants infested by aphids have earlier been found to induce a defence in other barley plants (Pettersson *et al.*, 1996). In this study, no changes were found regarding total dry weight (TDW) between barley cultivar Kara exposed to aphid infested barley cultivar Alva (A*K), Kara exposed to aphid infested Kara (K*K) and Kara exposed to Kara (KK) (table 1, appendix). This is the most interesting result of this study, since it indicates that the induced defence found by Pettersson *et al.* (1996) does not correlate with costs in terms of decreased growth. These results stand in contrast to the ones of Glawe *et al.* (2003) and Karban et al. (2004), who found costs associated with induced defence.

A*K shows a trend to allocate biomass from the leaves to the roots and stem. K*K and KK shows trends to allocate more biomass to the leaves (table 1).

From the results one could draw the conclusion that aphid infested Alva tend to have greater root length and less stem length than infested and not infested Kara. Infested Kara could be said to tend to increase its LAR. This could possibly depend on the different qualities of the cultivars. The conclusion can be made that aphid infestation does not lead to any major changes in growth or biomass allocation. Pettersson *et al.* (1996) has however shown that total root length (main root + side roots) increased in aphid infested barley plants. In this study only the main root was measured, and it is possible that the total root length would agree with the results of Pettersson *et al.*. Another explanation of the different results could be that the changes in biomass allocation in the study of Pettersson et al. (1996) are only temporary. The present study lasted for a longer period of time, and it can be argued that fluctuations in biomass allocation may have appeared, but results finally settled on values of the untreated control plants. Measurements were however taken continuously, and no such fluctuations have been seen.

In a preliminary study, where the same treatments (A*K, K*K and KK) were tested, leaf length of induced plants increased. In the however, plants grew in soil and no data for the roots was collected, nor for the weight of the plant parts. Therefore it is difficult to explain why this study and the preliminary study differ, since the experimental design of the two studies was very different. It is possible that the greater leaf length in the preliminary study was correlated with less root biomass, similar to what Ninkovic (2003) found. In the study of Ninkovic (2003) Kara plants allocated biomass to the roots when Alva induced Kara. It is also however possible that aphid infested Alva inducing Kara gives another reaction

than uninfested Alva inducing Kara. This indicates that the released volatiles change when Alva is aphid infested.

Potential sources of error

In this study, all treatments had similar effects. Thus, the root mass fraction was the same throughout the whole experiment, although at least the root mass fraction of the control should have decreased with time. That is what normally happens with a plant during development (Hunt, 1990).

One explanation for the results of both provoked and induced plants could be that the irrigation and nutrition system did not function satisfactory. The plants may have suffered from nutrient deficiency. When plants experience lack of nutrients photosynthesis decreases and biomass will be allocated to the roots (Poorter, 2002). This would explain why the root mass fraction in the present experiment did not decrease with time.

The nutrition problem can be seen as a systematic error, and is the most probable explanation to the unexpected results. In a situation when plants lack nutrients, it is uncertain if they will emit volatiles and respond to volatiles in the same way as a plant with sufficient nutrient supply would do. The reliability of the results can therefore be questioned.

Different experimental design?

Perhaps the changes in growth allocation are not dependent on how many aphids have infested the plants, but for how long they have been there. It takes about five days for the plants to induce a defence when exposed to volatiles from infested plants (Pettersson *et al*., unpublished). If the aphids had been on the plants for a longer period of time, it is possible that the results would have been different. There were however initial problems in the present study to establish the aphid populations on the plants. Instead of three- to eightfold increase the population per day, the number of aphids remained about the same the first week. This may have effected the production and response of plant volatiles. The number of aphids placed on each plant (five/plant) was chosen on basis of what would both simulate a severe attack during field conditions in the second leaf stage, but not cause too much damage to the plants.

It also takes time for receiving plant to respond to volatiles. Bean plants for instance exposed to MeJA need two days before they become attractive to a herbivore natural enemy (see review Dicke *et al.*, 2000). This argues for a longer term experiment.

The aphids had a tendency to escape from their assigned plants in the chambers, resulting in aphids feeding on the wrong plants. This happened despite precautions taken to avoid it, such as glue covered plastic rings around the infested plants.

Sample number four was most probably incorrect. Almost all values from sample four are extreme outliers. Therefore, the statistics were done without that sample. A probable explanation to the outlying values is observer error. Many of the results have an unacceptably high standard deviation, which makes the data and conclusions made upon them unreliable.

Experiment two:
Barley plants exposed to methyl jasmonate and methyl salicylate

No changes in biomass

Unpublished data from Pettersson *et al.* has shown that Kara plants exposed to volatiles from MeJA and MeSA induce a defence against aphids. In this study three treatments were carried out; Kara exposed to MeJA, Kara exposed to MeSA and Kara exposed to clean air. No differences between the treatments were seen regarding total dry weight (table 2, appendix). This indicates that the defence MeJA and MeSA induces in Kara plants does not lead to any decrease in growth.

The results could indicate that induced defence in plants does not lead to great costs for the plants in view of green plant biomass. Baldwin (1998) has shown that wild tobacco (*Nicotiana attenuata*) produced less seeds when exposed to MeJA in the absence of herbivores. Since plants in this study were not allowed to grow until seed production, the result does not necessarily stand in contradiction to that of Baldwin (1998).

Jasmonic acid leads to downregulation of genes encoding for proteins involved in photosynthesis (Creelman et al., 1997). Therefore, plants exposed to MeJA are suggested to experience less photosynthesis and decreased growth. Again, the present experiment failed to replicate this effect on growth.

Kara exposed to MeJA have significantly higher SLA than Kara exposed to MeSA and Kara exposed to clean air. Kara exposed to MeJA also tend to have higher TSW than Kara exposed to MeSA. MeSA-treated plants on the other hand have lower SLA than MJ and Kara exposed to clean air. 0K.

Low-SLA species have more cell wall compounds, especially lignin, and accumulate more soluble phenolics. These characteristics help the plant to defend itself from herbivores (Poorter, 2002). But it is not clear if a required low SLA in a plant gives the plant the same characteristics as low-SLA species. If this were the case, MeSA would induce a stronger defence in plants than MeJA, according to the results in experiment two.

Potential sources of error

Irradiance

Due to problems with the irrigation system in September, experiment two was conducted in October-November. The outdoor light is at that time of year not sufficient for the plants and artificial light was therefore added. Artificial light can however not fully imitate sunlight and plants tend to elongate more when growing under artificial light than under sunlight. The length of the stems and to some extent the leaves can therefore be expected to be larger than under natural conditions.

At decreasing irradiance plants have higher SLA and lower ULR. They also tend to have lower LMF (Poorter, 2002). In experiment two, artificial light was added, but it is possible that it was not sufficient. However, this cannot explain differences in SLA between the treatments, which all grew under the same conditions.

Seasonal effects

Light conditions aside, the late season when experiment two was conducted complicates the conclusions to be taken from the results. The barley cultivars that were used in the test are spring cultivars. During natural conditions they are not supposed to growing in late autumn. Even when growing in a greenhouse with accurate temperature and artificial light, it is possible that the plants do not react as normal because they are adapted for germination during spring. It is therefore suggested that more experiments be done during springtime.

Nutrition

Many plants in experiment two developed small necroses in the top of the leaves. This could indicate that they lack some nutrients, despite the continued watering with nutrients. One explanation for the nutrient deficiency could be that the nutrients poured through the sand. Under natural conditions, the nutrients stay in the soil for a longer time. The roots may have had difficulties to take up some of the nutrients before they were washed away to the draining pipe. As mentioned before, the behaviour of a plant lacking nutrients might not be the same as for a plant experiencing no nutrient deficiency. However, in experiment two, no other signs of nutrient deficiency than the small necroses were found. These necroses in themselves are not thought to have affected the result to a great extent.

Control

The treatment Alva exposed to Kara, AK, was added to experiment two in order to see if the conditions in the greenhouse were appropriate. Ninkovic (2003) has shown that AK allocates significantly more biomass to the roots and increases its SLA compared to 0K. In the present experiment though, no significant changes between AK and 0K was seen. This could indicate that the conditions in the greenhouse were not optimal, or that the results of Ninkovic (2003) were obtained during somewhat different experimental conditions.

Reflections on both experiments

Young plants react differently from older plants. Therefore laboratory experiments do not always give the same results as field experiments (Bruin *et al*., 2001). This has for example been found in alder tree experiments. Old alder trees are suggested to respond to a greater extent to volatiles than young alders (Dolch *et al.*, 2000). Therefore, the results from the experiments done in this study might have showed more significant changes if they had been conducted when the barley plants were older. This would however have necessitated a somewhat different experimental design. More replicates could possibly have made the results easier to interpret, but under present conditions it was not possible to conduct such experiment.

Final conclusions

The volatiles of the barley cultivars Kara and Alva have been shown to induce defence against aphids in neighbouring plants of a different cultivar (Pettersson *et al.* 1999). Now that no costs (=negative effects) have been observed when Kara and Alva are exposed to aphid infested plants, there is evidence to suggest that it would be interesting to mix the two cultivars Alva and Kara when sowing barley, in order to get a better defence against aphid infestations than barley field sown with only one variety. Both cultivars mature at the same time and give about the same yield (Olrog *et al.*, 1997). The ability to induce defence should however be weighed against other qualities of the cultivar, like yield, resistance against pathogens and ability to cope with other stresses, such as drought and cold.

According to the results from experiments two, barley plants of cultivar Kara exposed to MeJA or MeSA did not show any decrease in growth and no major changes in allocation of biomass.

In the introduction, two hypothesis to be tested were presented: Whether growth will be reduced in a barley plants exposed to volatiles released from other barley plants, attacked by aphids, and whether growth will be reduced in a barley plant when exposed to each of the volatile chemicals methyl salicylate and methyl jasmonate. The results of this study indicate that no reduction in growth occur in

either case. To my knowledge, no other published studies have produced same results as found here.

If the present results are shown to be repeatable, the future holds exiting possibilities of a more environmental friendly aphid control. There already exist products to control insects containing the harmless substance MeSA. With the knowledge that exposure to MeSA and MeJA t does not lead to any costs for the plant, these substances could become even more attractive for use in aphid control.

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APPENDIX

Tabel 1. *Results from experiment one. P-values, mean and standard deviation. A*K= Barley cultivar Kara exposed to aphid infestedbarley cultivar Alva, K*K = Kara exposed to aphid infested Kara, KK = Kara exposed Kara. T=Treatment, D=Days, T*D=Treatment *Days, d.f.=degrees of freedom.*

		Mean ± standard deviation			
Morphological parameters	**ANOVA**	**A*K**	**K*K**	**KK**	**d.f.**
SLA (m2/kg)	pT=n.s. pD=n.s. pT*D=n.s.	53,7[a] ± 4,5	52,3[a] ± 9,0	54,1[a] ± 5,5	2 3 6
LMF	pT=n.s. pD=n.s. pT*D=n.s.	0,37[a] ± 0,08	0,4[a] ± 0,07	0,39[a] ± 0,04	2 3 6
RMF	pT=n.s. pD=n.s. pT*D=n.s.	0,47[a] ± 0,08	0,43[a] ± 0,07	0,46[a] ± 0,04	2 3 6
SMF	pT=n.s. pD=0,000 pT*D=n.s.	0,16[a] ± 0,04	0,17[a] ± 0,04	0,16[a] ± 0,03	2 3 6
LAR (cm2/mg)	pT=n.s. pD=n.s. pT*D=0,027	0,21[a] ± 0,42	0,21[a] ± 0,05	0,21[a] ± 0,03	2 3 6
S:R	pT=n.s. pD=n.s. pT*D=n.s.	1,22[a] ± 0,41	1,37[a] ± 0,40	1,21[a] ± 0,21	2 3 6
TDW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	66,4[a] ± 34	61,5[a] ± 36,8	68,4[a] ± 43,8	2 3 6
TLW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	24,7[a] ± 13,7	24,3[a] ± 14,4	26[a] ± 15,8	2 3 6
TSW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	10,9[a] ± 7,2	9,8[a] ± 5,7	10,8[a] ± 7,9	2 3 6
TRW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	14,0[a] ± 7,82	12,4[a] ± 6,78	13,8[a] ± 7,77	2 3 6
TLW+TSW (mg)	pT=n.s. pD=0,000 pT*D=n.s.	0,036[a] ± 0,02	0,03[a] ± 0,02	0,037[a] ± 0,02	2 3 6

		Mean ± standard deviation			
Morphological parameters	**ANOVA**	**A*K**	**K*K**	**KK**	**d.f.**
Root length (cm)	pT=n.s. pD=0,000 pT*D=n.s.	32[a] ± 19,8	29,7[a] ± 16,7	27,1[a] ± 17,2	2 3 6
Stem length (cm)	pT=n.s. pD=0,000 pT*D=n.s.	8,0[a] ± 3,25	7,96[a] ± 1,99	7,06[a] ± 1,83	2 3 6
Leaf length (cm)	pT=n.s. pD=0,000 pT*D=n.s.	37,5[a] ± 20,1	34,7[a] ± 18,0	36,7[a] ± 20,3	2 3 6
Stem and leaf length (cm)	pT=n.s. pD=0,000 pT*D=n.s.	28,3[a] ± 9,7	27,4[a] ± 9,4	28,0[a] ± 9,7	2 3 6
Leaf area (cm2)	pT=n.s. pD=n.s. pT*D=n.s.	14,0[a] ± 7,8	12,4[a] ± 6,8	13,8[a] ± 7,8	2 3 6
Physiological parameters	**ANOVA**	**A*K**	**K*K**	**KK**	d.f.
RGR (g/g day)	pT=n.s. pD=0,000 pT*D=0,000	0,089[a] ± 0,05	0,07[a] ± 0,046	0,08[a] ± 0,05	2 3 6
ULR (g/m2 day)	pT=n.s. pD=0,003 pT*D=0,001	2,46[a] ± 2,10	1,92[a] ± 1,21	2,11[a] ± 1,35	2 3 6

Tabel 2. *Results from experiment two. P-values, mean and standard deviation. MS= Barley cultivar Kara exposed to methyl salicylate, MJ = Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. T=Treatment, D=Days, T*D=Treatment *Days, d.f=degrees of freedom.*

			Mean ± standard deviation		
Morphological parameters	**d.f**	**ANOVA**	**MS**	**MJ**	**0K**
SLA (m2/kg)	2 4 8	pT=0,005 pD=0,000 pT*D=0,000	$47,4^{a}$ ± 5,7	$52,8^{b}$ ± 15,6	$45,9^{a}$ ± 8,3
LMF	2 4 8	pT=n.s. pD=0,002 pT*D=n.s.	$0,43^{a}$ ± 0,06	$0,41^{a}$ ± 0,1	$0,41^{a}$ ± 0,07
RMF	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$0,38^{a}$ ± 0,1	$0,37^{a}$ ± 0,1	$0,39^{a}$ ± 0,1
SMF	2 4 8	pT=n.s. pD=0,445 pT*D=n.s.	0,19+ ± 0,03	$0,22^{a}$ ± 0,06	$0,20^{a}$ ± 0,04
LAR (cm2/mg)	2 4 8	pT=n.s. pD=0,0012 pT*D=0,025	$0,020^{a}$ ± 0,003	$0,021^{a}$ ± 0,007	$0,019^{a}$ ± 0,004
S:R	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$1,71^{a}$ ± 0,55	$1,86^{a}$ ± 0,75	$1,65^{a}$ ± 0,40
TDW (mg)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$51,5^{a}$ ± 22,2	$58,7^{a}$ ± 31,5	$51,6^{a}$ ± 19,6
TLW (mg)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$23,8^{a}$ ± 12,3	$23,6^{a}$ ± 12,6	$21,9^{a}$ ± 11,2
TSW (mg)	2 4 8	pT=0,012 pD=0,000 pT*D=n.s.	$9,61^{a}$ ± 3,57	$12,4^{b}$ ± 6,6	$10,3^{a}$ ± 4,0
TRW (mg)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$19,2^{a}$ ± 5,7	$20,2^{a}$ ± 8,3	$19,6^{a}$ ± 6,8
TLW+TSW (mg)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	$33,4^{a}$ ± 15,4	36^{a} ± 17,5	$32,2^{a}$ ± 13,9
Root length (cm)	2 4 8	pT=n.s. pD=0,002 pT*D=n.s.	24^{a} ± 9,2	$20,8^{a}$ ± 8,7	22^{a} ± 9,6

			Mean ± standard deviation		
Morphological parameters	**d.f**	**ANOVA**	**MS**	**MJ**	**0K**
Stem length (cm)	2 4 8	pT=n.s. pD=0,001 pT*D=n.s.	7,30[a] ± 1,0	7,52[a] ± 1,0	7,34[a] ± 1,3
Leaf length (cm)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	24,0[a] ± 9,2	20,8[a] ± 8,7	22,0[a] ± 9,6
Leaf area (cm2)	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	11,0[a] ± 5,3	11,3[a] ± 4,4	10,1[a] ± 5,3
Leaf area log	2 4 8	pT=n.s. pD=0,000 pT*D=n.s.	0,99[a] ± 0,21	1,02[a] ± 0,19	0,95[a] ± 0,22
Physiological parameters	d.f.	ANOVA	MS	MJ	0K
RGR (g/g day)	2 3 6	pT=n.s. pD=0,0005 pT*D=0,019	90,0[a] ± 63,6	97,1[a] ± 45,6	79,9[a] ± 60,9
ULR (g/m2 day)	2 3 6	pT=n.s. pD=0,000 pT*D=0,003	2,77[a] ± 2,0	2,88[a] ± 1,40	2,62[a] ± 2,0

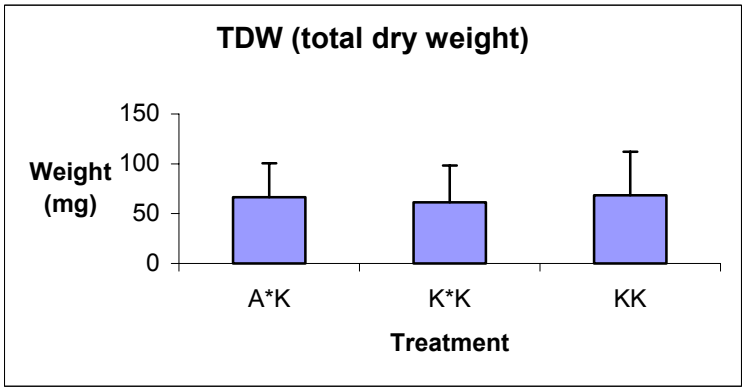

Figure 1. *TDW of experiment one. Mean values and standard deviation. A*K= Barley cultivar Kara exposed to aphid infestedbarley cultivar Alva, K*K = Kara exposed to aphid infested Kara, KK = Kara exposed Kara. No significant differences or trends shown between the treatments.*

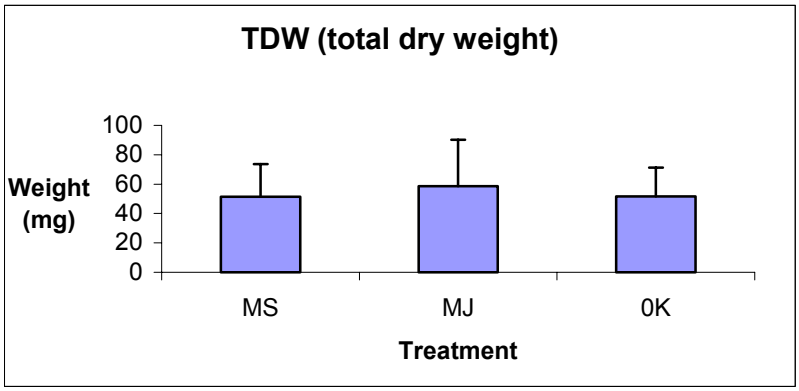

Figure 2. *TDW of experiment two. Mean values and standard deviation. MS= Barley cultivar Kara exposed to methyl salicylate, MJ = Kara exposed to methyl jasmonate, 0K = Kara exposed to clean air. No significant differences or trends shown between the treatments.*