Volatile mediated plant-plant communication in cultivar mixtures:

From plant to aphid responses

Iris Dahlin
Faculty of Natural Resources and Agricultural Sciences
Department of Crop Production Ecology
Uppsala

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Abstract

Modern agriculture is characterized by simplified cropping systems, associated with negative effects on environment, biodiversity and biological control potential. Cultivar mixtures are often presented as a more efficient and sustainable alternative due to additive effects of cultivar properties and pest reduction, but previous studies have provided equivocal results for this claim. Therefore, a better understanding of the processes underlying the effects of crop genotypic diversity on plant and herbivore responses is needed.

Our review of published studies on volatile interactions between plants demonstrates that plant communication is a general event not limited to damaged plants, and that it is more a phenomenon of volatile eavesdropping rather than signalling. Empirical studies in this thesis show that barley plants perceive the growth pattern of their neighbours via volatiles and adapt their own growth strategy accordingly, which can be seen as a preparation for future competition. Field experiments show different trait responses of cultivars grown in mixtures, and that these responses depend on the neighbouring cultivar identity. Plants responded to their neighbours with adaptive and maladaptive growth responses, with increased nitrogen accumulation efficiency, biomass production and grain yield as a result. Aphid populations were generally supressed in these cultivar mixtures; not due to aphid colonization pattern or natural enemy abundance, but possibly induced by volatile interactions between neighbouring plants of different cultivars. Aphid responses to one cultivar in a mixture were neighbour specific and pest suppression was most pronounced when both cultivars in a mixture showed a reduced aphid-plant acceptance after reciprocal volatile exposure in the laboratory. Such individual neighbour responses mediated by volatiles can explain the inconsistent effects of cultivar mixtures in previous research. The findings of this thesis establish a better understanding of volatile communication between plants adding a new dimension to plant behaviour and community processes. Combining cultivars in mixtures based on how they interact with each other is a promising strategy for productive and sustainable agriculture.

Keywords: biodiversity, future competition, growth pattern, pest management, plant-insect interaction, plant plasticity, plant trait, productivity

Author's address: Iris Dahlin, SLU, Department of Crop Production Ecology,
P.O. Box 7043, 750 07 Uppsala, Sweden

Växt-växtkommunikation genom flyktiga ämnen i sortblandningar: Från växt till bladlusreaktioner

Abstrakt

Modernt jordbruk präglas av förenklade odlingssystem, förknippade med negativa effekter på miljö, biologisk mångfald och biologisk kontrollpotential. Sortblandningar presenteras ofta som ett effektivare och mer hållbart alternativ på grund av additiva effekter av sortegenskaper och minskning av skadegörare, men tidigare studier gav tvetydiga resultat avseende dessa påståenden. Därför behövs en bättre förståelse av de processer som ligger till grund för effekterna av genotypisk mångfald av grödor på reaktioner av växt- och skadegörare.

Vår genomgång av publicerade studier om växtinteraktioner genom flyktiga ämnen visar att växtkommunikation är en allmän händelse som inte är begränsad till skadade växter, och att det är snarare frågan om tjuvlyssning än signalering. Empiriska studier i denna avhandling har visat att kornplantor uppfattar tillväxtmönstren av sina grannar via flyktiga ämnen och anpassa sin egen tillväxtstrategi därefter, vilket kan ses som en förberedelse för framtida konkurrens. Fältförsöken visar olika respons i växtegenskaper av sorter som odlades i blandningar, och att responsen beror på identitet av sorter i närheten. Växter reagerade på sina grannar med adaptiv och maladaptiv tillväxt, med ökat kväveackumuleringseffektivitet, biomassaproduktion och avkastning som följd. Bladluspopulationer undertrycktes i allmänhet i dessa experiment; inte på grund av bladlössens koloniserings mönster eller antalet naturliga fiender, men möjligen inducerat av interaktioner mellan kombinerade sorter genom flyktiga ämnen. Bladlus responsen på en sort i en blandning var grannspecifik och skadedjursbekämpning var mest uttalad när båda sorterna i en blandning visade en minskad växtacceptans efter ömsesidig exponering till flyktiga ämnen i laboratoriet. Sådana individuella reaktioner till grannar som medierats av flyktiga ämnen kan förklara de inkonsekventa effekterna av sortblandningar i tidigare forskning.

Resultaten av denna avhandling ger en bättre förståelse av växtkommunikation genom flyktiga ämnen, vilket lägger till en ny dimension till växtbeteende och samhällsprocesser. Att kombinera sorter i blandningar baserat på hur de interagerar med varandra är en lovande strategi för produktivt och hållbart jordbruk.

Nyckelord: biologisk mångfald, framtida konkurrens, produktivitet, skadedjursbekämpning, tillväxtmönster, växtegenskaper, växt-insekt interaktion, växtplasticitet

Dedication

To my wonderful family.

It is not the strongest of the species that survive, nor the most intelligent, but the one most responsive to change.
Charles Darwin

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ninkovic, V, Markovic, D & Dahlin, I (2016). Decoding neighbour volatiles in preparation for future competition and implications for tritrophic interactions. *Perspectives in Plant Ecology, Evolution and Systematics* 23, pp. 11-17.

II Dahlin, I, Markovic, D, Glinwood, R, Rubene, D & Ninkovic, V (2019). Plants perceive the growth pattern of their neighbors through volatiles and adapt their own growth strategy accordingly (manuscript).

III Dahlin, I, Kiær, LP, Bergkvist, G, Weih, M & Ninkovic, V (2019). Plasticity of barley in response to plant neighbors in cultivar mixtures (submitted).

IV Dahlin, I, Rubene, D, Glinwood, R & Ninkovic, V (2018). Pest suppression in cultivar mixtures is influenced by neighbour-specific plant-plant communication. *Ecological Applications* 28, pp. 2187-2196.

Papers I and IV are reproduced with the permission of the publishers.

The contribution of Iris Dahlin to the papers included in this thesis was as follows:

I Co-author. Contributed in conceiving ideas, literature search and writing the review.

II First author. Conceived the ideas and design methodology together with VN and DM. Performed lab work in cooperation with DM. Wrote the paper with revisions of co-authors. Will be responsible for interaction with reviewers once the paper is submitted.

III First author. Conceived the ideas and design methodology together with the co-authors. Planned, performed and oversaw fieldwork. Wrote the paper in cooperation with co-authors. Responsible for interaction with editors and reviewers.

IV First author. Designed together with VN the methodology. Planned, performed and oversaw lab- and fieldwork. Collected together with RG plant volatiles. Contributed equally to this work with DR as authors and responded to comments from reviewers.

1 Introduction

Agricultural development in the recent decades has led to cultivation of crop fields in pure stands with almost genetically identical plants (Potts et al., 2010; FAO, 2011; Dudley and Alexander, 2017). Plants with the same nutritional requirements have to compete for shared resources. Insect pests and diseases can spread easily from plant to plant if all plants are susceptible to the same pest species. These simplified agricultural systems are associated with negative effects of fertilizer application on soil, air, and water quality (Lal, 2008; Sutton et al., 2011), and negative effects of pesticides on non-target organisms (Gill et al., 2012; Hallmann et al., 2014), biodiversity and biological control potential (Flynn et al., 2009; Geiger et al., 2010; Oliver et al., 2015). We need to develop new, sustainable and efficient systems to reduce the environmental impact of modern agriculture (Tilman et al., 2002; Foley et al., 2011).

1.1 Cultivar mixtures

Diverse plant species mixtures (i.e. intercropping) have been studied as an integrated pest management tool for their potential to improve pest suppression (Landis et al., 2000; Gaba et al., 2015). Although the strategy has been effective against pests, it has not been implemented in developed countries due to high costs and technical difficulties (Lin, 2011). An easier way to introduce biodiversity in agriculture is to grow mixtures of cultivars within a species (Butler et al., 2007), which could improve system resilience (Bullock et al., 2017). The value of such a system compared to crops grown in pure stands has been demonstrated in empirical studies by promoting productivity (Cook-Patton et al., 2011; Tooker and Frank, 2012; Prieto et al., 2015; Reiss and Drinkwater, 2017), herbivore suppression (Shoffner and Tooker, 2013; Zytynska et al., 2014), arthropod richness (Crutsinger et al., 2006) and disease management (Mundt, 2002; Cox et al., 2004).

However, the effectiveness of this system appears inconsistent; some examples indicate no effects, or even increased herbivore abundance in cultivar mixtures (Hambäck et al., 2010; Utsumi et al., 2011; Dubs et al., 2018). The same inconsistencies have been found in terms of productivity (Reiss and Drinkwater, 2017; Borg et al., 2018). Therefore, a better understanding of the processes underlying the effects of crop genotypic diversity on plant and herbivore responses is needed to explain why some studies find positive effects whereas others do not, and to enable predictive development of sustainable agricultural cropping systems based on genotype diversity.

In this project, we aimed to discover the mechanisms underlying this inconsistency. Are plant interactions the main cause of inconsistency in mixture effects? Are such interactions between plants induced by responses to volatile cues? The answers will fill knowledge gaps, making cultivar mixtures a more reliable tool, used to a greater extent by farmers, to obtain a productive and sustainable agriculture system.

1.2 Plant-plant interactions

Plants cannot escape unfavourable locations, conditions, or threats from superior competitors and herbivores, because they are rooted. The most common and serious challenge that plants are exposed to is resource competition from co-occurring plants. In plant communities, individual plants can affect each other by competing for resources such as light, water, nutrients, or space. Further, each plant might interact chemically with its neighbour by the release of chemical compounds, which can have direct or indirect influence on surrounding organisms in a positive, negative or a neutral manner (Rice, 1984). Nevertheless, plants are not defenceless; they sense their environment for signals, and respond by adjusting their phenotype accordingly (Trewavas, 2005; Gratani, 2014; Turcotte and Levine, 2016).

Volatile signals are among the crucial cues, because they can be perceived early in a plants lifetime, for example before being shaded or coming into contact with their neighbours. Moreover, plant volatiles carry detailed information on the identity and physiological condition of the emitter (Knudsen and Gershenzon, 2006; Karban et al., 2014; Dicke, 2015). If a superior competitor is detected, plants can respond with phenotypic plasticity through physiological and morphological changes increasing their fitness (Violle et al., 2009; Cahill and McNickle, 2011) even before competition takes place (Kegge et al., 2015; Novoplansky, 2016).

Plant volatile emissions can change through induction of both biotic and abiotic stress factors, such as herbivore attack, high temperature or light intensity (Callaway et al., 2003; Holopainen and Gershenzon, 2010). The ability to respond to such environmental cues is genetically based (Via and Lande, 1985; Norouzitallab et al., 2019), varying between crop cultivars and environmental stimuli. Herbivore induced plant volatiles are among the best-studied plant responses to stress, and may ‘warn’ undamaged neighbours of attack (Dicke and Baldwin, 2010). Such herbivore induced volatiles can directly activate resistance-related genes (Heil and Karban, 2010) or prime eavesdropping plants for a stronger and faster induction of defence when attacked (Heil and Ton, 2008; Mauch-Mani et al., 2017).

So far, volatile communication between plants has not been considered in investigations of plant responses to their neighbours in cultivar mixtures under field conditions. Shoffner and Tooker (2013) found that wheat (*Triticum aestivum*) mixtures and monocultures grown in greenhouse chambers emitted the same volatile compounds, but mixtures emitted greater amounts than monocultures. This is in line with the study of Ninkovic and colleagues (2013), who found consistently greater quantities of two terpenoids in the headspace of potato previously exposed to volatiles from onion plants, which suggests physiological changes in exposed plants. However, changes in the volatile profiles of exposed plants do not necessarily have to be due to a physiological change; neighbour-emitted volatiles can also be adsorbed to the foliage of exposed plants then re-released (Himanen et al., 2010).

Herbivore insects can be very sensitive to slight changes in their host plants, thus when visual, chemical or nutritional cues change, (i.e. due to plant neighbour responses) herbivore behaviour and abundance can be affected (Dicke, 1994; Powell et al., 2006; Ninkovic et al., 2013). For example, when potato plants were exposed to volatiles from onions, their volatile profile changed, leading to avoidance by green peach aphids (*Myzus persicae* L.) in laboratory experiments (Ninkovic et al., 2013; Dahlin et al., 2015). Further, volatile induced responses have been shown to affect aphid-plant acceptance in different genotypes of the same species (Ninkovic et al., 2002), and this was correlated with reduced aphid growth (Ninkovic and Åhman, 2009).

Although many studies have reported phenotypic plasticity in response to plant neighbours of different species (Abakumova et al., 2016), little is known about phenotypic plasticity in cultivar mixtures and whether these are adaptive.

Further, very little is known about phenotypic growth responses induced by plant neighbour volatiles and how this might affect aphids.

1.3 Model system

The model system chosen for this thesis consists of interactions between barley (*Hordeum vulgare* L.) plants of different cultivars, and their effects on an insect herbivore and plant pest, the bird cherry-oat aphid *Rhopalosiphum padi* (L.).

1.3.1 Plant - barley

Barley belongs to the grass family Poaceae and is a major cereal crop in temperate climates globally. Barley has been used as animal fodder, in the production of beverages and as human food. Wild barley, *H. spontaneum*, is considered the ancestor of domesticated barley (von Bothmer and Komatsuda, 2010) and possesses different genes, alleles and regulators with the potential for resistance to environmental stresses (Wang et al., 2018). This property of wild barley has been used to breed for aphid-plant resistance - an alternative to chemical control - and barley cultivars have been bred for resistance to the Russian wheat aphid (*Diuraphis noxia*) and greenbug (*Schizaphis graminum*) (Mornhinweg et al. 2012). However, even though cultivar differences in susceptibility to aphids exist, a barley cultivar completely resistant to *R. padi* has not been found yet (Åhman and Bengtsson, 2019). In addition, aphids can overcome resistant cultivars when resistance-breaking biotypes emerge (Smith and Chuang, 2014; Thomas et al. 2016).

1.3.2 Herbivore - aphids

Rhopalosiphum padi is a host-alternating aphid species, which hibernates in the winter as eggs on its primary host the bird cherry (*Prunus padus* L.). After the eggs hatch and one or two wingless generations are completed, winged aphids develop that migrate to grasses and cereals in the spring (secondary hosts), producing wingless offspring (Fig. 1). In late summer, winged aphids are born in response to crowding and decreased plant quality, migrate to grasses and return to the primary host in autumn (Dixon, 1971; Wiktelius et al., 1990).

Figure 1. Oat aphids on bird cherry and on barley. (Photos by N. Bridge CC BY SA 3.0 and S. Al Abassi, SLU.)

To maximize survival and reproduction, it is necessary for aphids to distinguish between good host and poor/non-host plants, which involves their sensory system (Smith, 2005). Finding appropriate host plants and feeding sites is a stepwise procedure with a range of different stimuli including visual, chemical, tactile and nutritional cues (Powell et al., 2006).

For instance, landing of winged *R. padi* is elicited by visual stimuli (Åhman et al., 1985; Doring and Chittka, 2007). Most aphids will preferentially land on yellow surfaces, but it has been shown that *R. padi* are most responsive to light green wavelengths (Nottingham et al., 1991). Olfactory cues (i.e. plant volatiles) can be important in host finding and the initiation of probing for both winged and wingless morphs of *R. padi* (Pickett et al., 1992; Quiroz and Niemeyer, 1998; Pettersson et al., 2007). Non-volatile tissue parameters can be detected through contact chemoreceptors on the antennae (Powell et al., 2006). During probing, small samples of plant sap are ingested and tasted, and some feeding stimuli are associated with the quality of plant sap, which is related to content and composition of amino acids (Sandström, 1998). Thus, aphid plant acceptance is affected by the physical appearance, odour and diet quality of its host plant (Dixon and Kindlmann, 1998).

Aphids have a high capacity for reproduction (Awmack and Leather, 2002); under optimal conditions, an individual aphid starts reproduction 7 to 10 days after it is born (Dixon and Kindlmann, 1998). *Rhopalosiphum padi* colonizes cereal fields during a concentrated migration period of about two weeks and reaches peak population size already after two generations, which takes about 20 to 30 days (Wiktelius, 1982). Hence, aphids are important agricultural pests and *R. padi* is a key pest of small grain cereals in temperate regions worldwide (Blackman and Eastop, 2007). Aphids cause direct damage to the crop by feeding on plant phloem sap or indirectly by the transmission of plant viruses, reducing crop yields (Mornhinweg, 2010; Jarosova et al., 2016). Aphid feeding causes economic damage in outbreak years with up to 60% yield reduction in cereals (Riedell et al., 2003) and aphid control is heavily dependent on prophylactic or acute use of insecticides with environmental impact such as negative effects on non-target organisms (Pimentel et al., 1992; Goulson, 2013). Stronger regulations on pesticide use, aphid resistance to pesticides and the demands of larger ecologically grown areas in Europe reduces access to efficient treatments against aphids, and poses great challenges for farmers. This makes the development of alternative control strategies against herbivores necessary (FAO, 2011; Dewar and Foster, 2017).

1.4 Aims of the thesis

The overall goal of the thesis was to investigate if the introduction of botanical diversity in agricultural fields through cultivar mixtures affects crop efficiency and aphid performance in terms of plant productivity and pest suppression.

The specific aims were to:

- Review the literature on volatile interactions between plants and its effect on higher trophic levels (paper I).
- Study plant growth responses of a cultivar, when exposed to volatiles from cultivars with different growth strategies (paper II).
- Determine if a cultivar shows different phenotypic responses in plant traits when grown together with another cultivar, and if this varies depending on the component cultivars (paper III).
- Examine if aphid-plant acceptance of one cultivar previously exposed to volatiles from another is reduced, and if aphid population development is suppressed on plants of one cultivar when grown together with another cultivar (paper IV).

2 Material and methods

We used a model system consisting of different spring barley cultivars and the bird cherry-oat aphid to test plant and aphid responses to volatile mediated interaction between plants. The barley cultivars used (Anakin, Fairytale, Luhkas Rosalina and Salome), were commercially available in Sweden during the project period. Test aphids for laboratory experiments were taken from a multi-clonal population grown in a greenhouse chamber.

In the laboratory, we collected and analysed volatile profiles of the barley cultivars, tested plant adaptive responses to their neighbours and examined aphid plant acceptance of volatile treated plants. In field experiments in two years, with barley cultivars grown in mixtures and in pure stands, we investigated variation in plant traits and aphid responses.

2.1 Plant exposure system

One of our aims was to study the effects of communication between plants on plant growth and aphid performance. To ensure that the test plants could only interact via volatile substances, we used a series of transparent two-chamber cages in which all other types of interaction (i.e. competition) were prevented (Ninkovic et al., 2002). The series of two-chamber cages (in total 84 cages) were placed in blocks in a 40 cm^2 growing chamber on two benches on each side of the room. Volatiles from barley plants in the inducing chamber follow the airflow to barley plants in the receiving chamber (Fig. 2), which were then used for growth measurements or aphid plant acceptance tests. For control plants, the inducing chamber was left empty. The exposure system has a large capacity, i.e. many cultivars can be tested simultaneously, all plants were treated under the same abiotic conditions (i.e. automatic water supply), and the cages could be adjusted to plant height during longer exposure periods. A more detailed description of the method is given in papers II and IV.

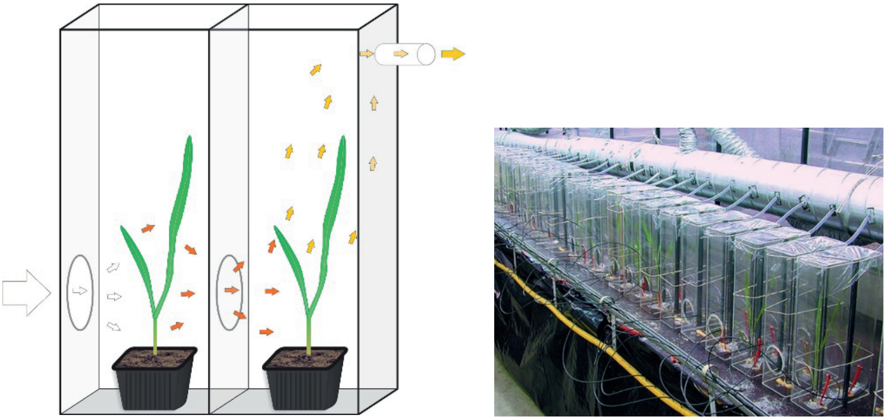

Figure 2. Exposure system (Artwork: D. Markovic, SLU. Photo: V. Ninkovic, SLU).

2.2 Plant volatile collection and analysis

Because plant growth and aphid performance on barley plants differed after volatile exposure of one barley cultivar to certain different cultivars, we hypothesised that there were differences in the volatile profiles of the emitting cultivars that the exposed plants responded to. Therefore, we collected and identified volatiles from the headspace of all inducing barley cultivars to examine any dissimilarities between them in volatile composition.

The collection system for volatile compounds used a push/pull technique and has been described in detail in Appendix 2 of paper IV. Briefly, plants were enclosed in oven bags, in which air was pushed in through charcoal filters and pulled out together with plant volatiles that were adsorbed onto a porous polymer (Porapak) (Fig. 3) from which they were extracted using a solvent. Volatile compounds were analysed using a gas chromatograph coupled to a mass spectrometer and identified by comparison of mass spectra with a commercial mass spectral library (NIST 2008) and authentic chemical standards where available (Glinwood et al., 2011). Volatiles were collected during 72 h to obtain enough for analysis and because the composition of plant volatiles has been shown to follow diurnal patterns (Loughrin et al., 1994).

We analysed the chemical composition of the volatile profiles of the cultivars and the differences in amounts and frequencies of individual chemical compounds.

Figure 3. Collection of plant volatiles. (Photo: R. Glinwood, SLU.)

2.3 Plant neighbour adaptation test

The aim of this experiment was to determine if a barley cultivar would change its growth when exposed to volatiles of cultivars with contrasting growth strategies. We exposed the cultivar Fairytale to volatiles from Fairytale (self-exposed control), Salome and Luhkas (slow, intermediate and fast growing respectively) separately, and evaluated traits of plants in the inducing and receiving chambers after 15 and 25 days of exposure. Due to the long exposure period and resulting plant size, the exposure cages were adjusted in height. Plant traits of leaves, stems and roots were measured, and the leaf-, stem- and root mass fractions (LMF, SMF and RMF) were calculated to analyse biomass distribution i.e. the dry mass of the respective plant organ per total plant dry mass. The leaves and roots were scanned to quantify leaf area, root length and volume.

We analysed plant trait differences between the inducing and between the responding plants with generalized linear models. Further, each exposed plant was paired with its inducer in the dataset to analyse pairwise correlations of the inducing effect on traits of the exposed plants. The methods are described in detail in paper II.

2.4 Aphid-plant acceptance test

With this experiment, we aimed to test if the effects of volatile exposure between barley plants can extend to the next trophic level and affect plant-aphid interactions. A no-choice aphid-settling test with minimal disturbance of the plants was performed immediately after the end of the exposure of one barley cultivar to volatiles of another (Ninkovic et al., 2002). A plastic tube was placed over the second leaf of the plants (Fig. 4) and 10 randomly chosen wingless aphids introduced. The number of aphids settled (not moving) on each leaf was recorded after two hours, which is sufficient time for aphids to settle and reach the phloem with their stylets (Prado and Tjallingii, 1997). With this no-choice method, all aphids eventually have to accept the test plant, which makes the test less sensitive than a test in which aphids can choose more than one feeding site, where aphids would probably test all choices and may not have chosen the most acceptable plant at the end of the test. The barley plants were tested at the second-leaf stage, the same phenological stage as during aphid colonization (i. e. when plant acceptance is of importance), to simulate the establishment phase, because plant acceptance is a key factor in the progress of an aphid infestation (Pettersson et al., 2007).

Differences in aphid-plant acceptance of cultivars exposed to volatiles from other cultivars and unexposed cultivars were analysed with generalized linear mixed models. Details are given in paper IV.

Figure 4. Aphid-plant acceptance test. (Photo: V. Ninkovic, SLU.)

2.5 Field experiments

Two field experiments were conducted at Lövsta field research station, Swedish University of Agricultural Sciences, in central-eastern Sweden in 2015 and 2016. Aphid occurrence was very low in 2015 and was not analysed in this year. The five spring barley cultivars used (Table 1) were grown in pure stands and in two-cultivar combinations in alternate rows, enabling cultivar identification of individual plants for measurements and sampling. By this method, we were able to evaluate plant and aphid responses at cultivar level rather than stand level. The cultivar Salome was chosen as a component in all mixtures because it affected aphid plant acceptance of the greatest number of volatile exposed cultivars in the laboratory (paper IV).

Table 1. *The cultivars of spring barley used in the field experiments.*

Cultivar	Breeder	Pedigree	Type	Maturity (d)	Yield index*	Plant height (cm)†
Anakin	Sejet Plant Breeding, DK	Tumbler x Respons	Fodder	110	103	70
Fairytale	Sejet Plant Breeding, DK	Colston x (Recept x Power)	Fodder	110	101	71
Luhkas	R.A.G.T. Seeds Ltd, UK	Annabell x Prestige	Fodder	108	101	68
Rosalina	Sejet Plant Breeding, DK	Beatrix x Eskobar	Malt	109	99	68
Salome	Nordsaat Saatzucht GmbH, DE	Auriga x (Publican x Beatrix)	Malt	109	104	64

*percentage relative to a reference mixture of cultivar Prestige, Justina, Orthega and Gustav.

†multi-year average (Larsson et al., 2013).

The seeds were sown in the beginning of May with 400 seeds m^2 and a row spacing of 12.5 cm. Each of the nine treatment plots (3 x 9 m) represented a replicate and were randomly placed in each of six blocks in a conventional randomized block design, with a distance of 1 m between the plots. The space between plots was unsown and regularly weeded by hand (Fig. 5).

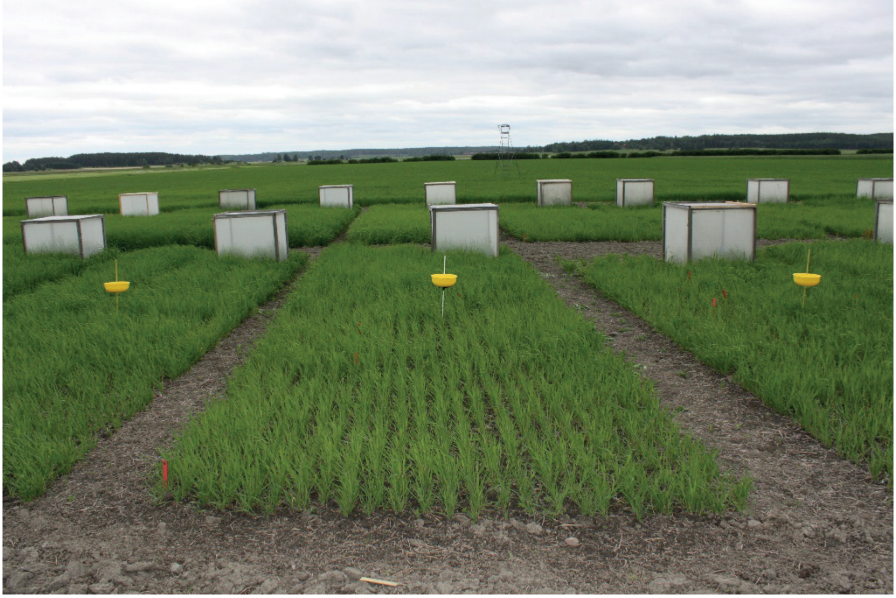

Figure 5. Spring barley cultivars grown in mixtures and pure stands. (Photo: D. Markovic, SLU.)

2.5.1 Plant trait responses

The aim of these field experiments was to determine if a specific cultivar shows different phenotypic responses in functional traits when grown together with another cultivar, and if this varies depending on the component cultivars.

A range of plant traits were assessed in each plot, covering plant development, growth and vigour, stretching, nitrogen economy and reproduction. Phenotypic responses to neighbouring plants are dynamic; therefore, we measured plant traits on different occasions to identify developmental adaptations during the growth of barley plants in response to plant neighbourhood.

When several plants were subsampled within a plot, they were considered as randomly selected representatives of any plant growing in the stand and were considered as the experimental unit for statistical analysis. We analysed overall differences in plant traits (i.e. of any cultivar), and those of individual cultivars, between mixtures and pure stands. Further, we analysed trait differences of cultivars grown together with another cultivar in general, and such differences for cultivar Salome (i.e. cultivar x with cultivar y, and Salome with cultivar y). We also analysed if plant traits became more similar when cultivars grew in a mixture than when grown in pure stands. Finally, plant traits were combined in functional groups and analysed for differences between diversity level (mixture versus pure stand), cultivars, and their interactions for the individual experimental years and for both years together. Details of the methods are given in paper III.

2.5.2 Aphid population development

The aim of this experiment was to determine if aphids and their natural enemies behave differently when cultivars were grown in mixtures or in pure stands. We assessed aphid immigration and population development, as well as the activity density of predatory arthropods, in the same field experiments described above.

Aphid abundance was determined by recording the number of aphids on three randomly chosen 1 m transects (Fig. 6) for each cultivar and plot, and summing these three values (Ninkovic et al., 2003). Aphid immigration to the field was measured using yellow water-traps and the activity density of predatory arthropods, such as ground-dwelling carabids and spiders, was determined using pitfall traps. In addition, two observers, one on each half of the plot to avoid recording the same individual twice (Ninkovic et al., 2011), simultaneously estimated the occurrence of the sevenspotted ladybird *Coccinella septempunctata*.

We estimated aphid population development in cultivar mixtures and cultivars grown in pure stands at the final population size and when half of the population size was reached. We analysed whether final population size depended on the population growth earlier in the season. Further, we analysed the effect of predator abundance on aphid population size, and the effect of cultivar mixture and aphid numbers on predator abundance. Details of the methods are described in paper IV.

Figure 6. One meter transects marked with red sticks and a yellow water-trap in a mixture of two barley cultivars. (Photo: I. Dahlin, SLU.)

3 Results and discussion

3.1 Plant-plant communication (paper I)

Airborne communication between plants is a subject that is involved in all four papers of this thesis. Plants mediate information via volatile organic compounds. In our exposure system, the information exchange is one-way, directed by the airflow in the system, i.e. information is mediated by volatiles from the emitter to the receiver (paper II and IV) and not vice versa. In the field, the exchange of information proceeds in both directions, neighbouring plants are both emitters and receivers (paper III and IV). Further, in our laboratory experiments, plants could solely communicate via volatiles. In the field, plants can also perceive other cues, which makes it difficult to distinguish which signals plants responded to (which is especially true for paper III).

Paper I provides a review of the literature on plant-plant interactions, and their effects on other organisms such as herbivores and their natural enemies, focusing on the role of volatiles. The reviewed studies provided examples of changes in plant volatile profiles due to biotic stressors, which coexisting plants respond to, increasing their fitness. This research has been going on for the last four decades, and is mostly related to damage-induced resistance of plants induced by volatiles, and their herbivore repelling and natural enemy attracting effects.

Other reviews on this topic (e.g. Dicke and van Loon, 2000; Chen, 2008; Heil, 2014) centred on priming of plants' indirect defence, induced by volatiles from herbivore-infested plants and having multitrophic effects. Our review also gives an overview of studies on volatile interactions between undamaged plants. With this, we broadened the perspective, showing much wider implications and thus drawing the attention from entomologists to ecologists, agronomists and other researchers in the field of plant science. Plant volatiles carry not only information on whether the emitter is being attacked, but also about its identity and physiological status. For example, the parasitic plant golden dodder (*Cuscuta pentagona*) could discriminate between volatiles of different neighbouring species, growing towards its host rather than a non-host in controlled experiments (Runyon et al., 2006). Moreover, a barley cultivar responded with shade-avoidance growth pattern, when exposed to volatiles from plants grown in low light conditions but not when the emitter plants were grown in normal light (Kegge et al., 2015). Plant scientists need to be aware, when they evaluate their experiments, that their test plants might grow differently in the neighbourhood of others.

Further, it seems that many of the cited authors in our review consider that the emitter plant has the intention to inform its neighbours, using wordings like "cry for help" (Dicke and Baldwin, 2010), "talking trees" (Baldwin et al., 2006) and "manipulating" (Bottrell et al., 1998; Dicke et al., 1990) in their titles. When a plant is attacked, it responds with induced resistance and such responses are usually expressed systemically to protect other yet undamaged parts of the plant. This within-plant signalling is mediated through vascular long-distance signals (Notaguchi and Okamoto, 2015), but also through volatile cues, which are transmitted faster to undamaged parts (Frost et al., 2007). Therefore, it is more a phenomenon of neighbour eavesdropping than signalling (Heil and Karban, 2010). The same applies to untacked plants. There is no obvious benefit for a plant to reveal if it is a host for parasitic plants, nor to reveal its competitiveness to neighbouring plants. The release of volatiles as physiological by-products cannot be turned off. On the contrary, a high physiological activity such as fast growth of a putative competitor (or biochemical induction due to infestation) may actually boost emissions (Dudareva et al., 2004) through higher respiration rates, changing the volatile profile, which can initiate specific growth responses that increase the competitive capacity of eavesdropping neighbours.

Plant communication is a general phenomenon and is not limited to damaged plants, a point that is slowly being recognised. For example, in a recent paper, Kigathi et al. (2019), found that plant volatile emission depends on neighbouring plants and not only on herbivore damage, but still focus on host searching insects and not on the impact on the responding plants themselves, which I will discuss in the next section.

3.2 Plant growth adaptation (paper II)

The cultivars involved in this part of the study (Luhkas, Salome and Fairytale) had contrasting growth strategies (fast, intermediate and slow). For most measured plant traits, cultivar Luhkas had significantly higher values than the other cultivars, followed by Salome and Fairytale at early growth stages (some of the traits in Fig. 7). Exceptions to this pattern were observed for Salome, which had the highest SLA and leaf area values, and for Fairytale, with the highest leaf- and root mass fraction.

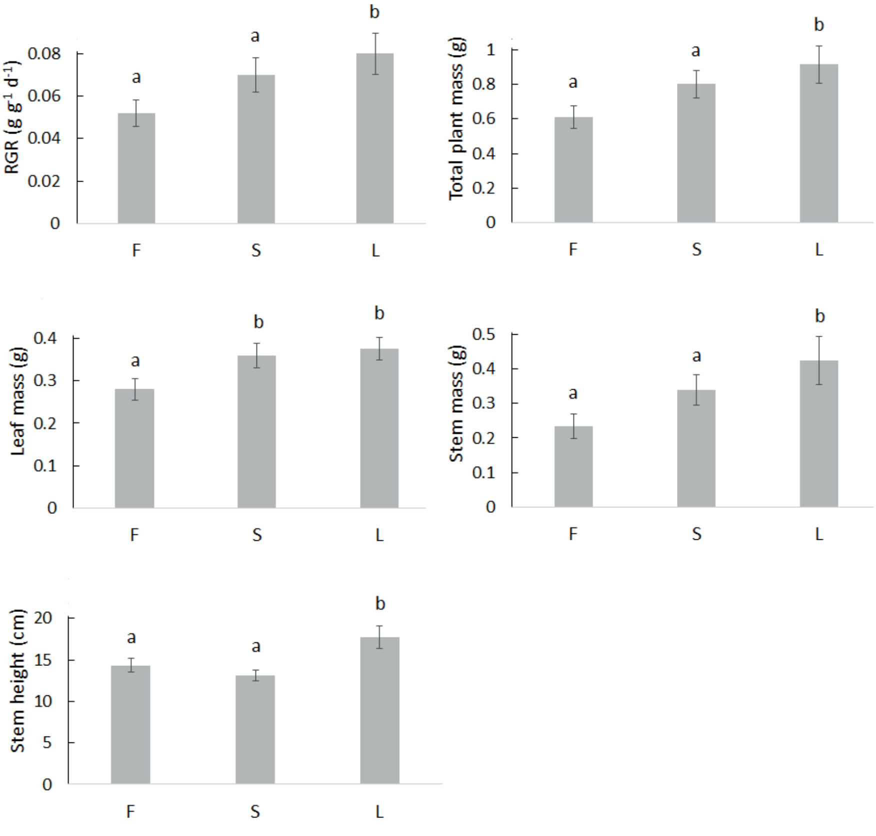

Figure 7. Mean plant trait values of the inducing cultivars Fairytale (F), Salome (S) and Luhkas (L), 25 days after sowing. Significant differences ($P < 0.05$) are indicated by different letters.

The growth strategy of Fairytale changed in accordance with the growth pattern of the cultivar whose volatiles it was exposed to. Fairytale exposed to Luhkas had the highest values for plant-, leaf- and stem biomass, stem height and relative growth rate compared to self-exposed Fairytale (Fig. 8). This shows that volatiles carry information about the growth development of their emitters, which induced receivers to adapt their own growth strategy accordingly.

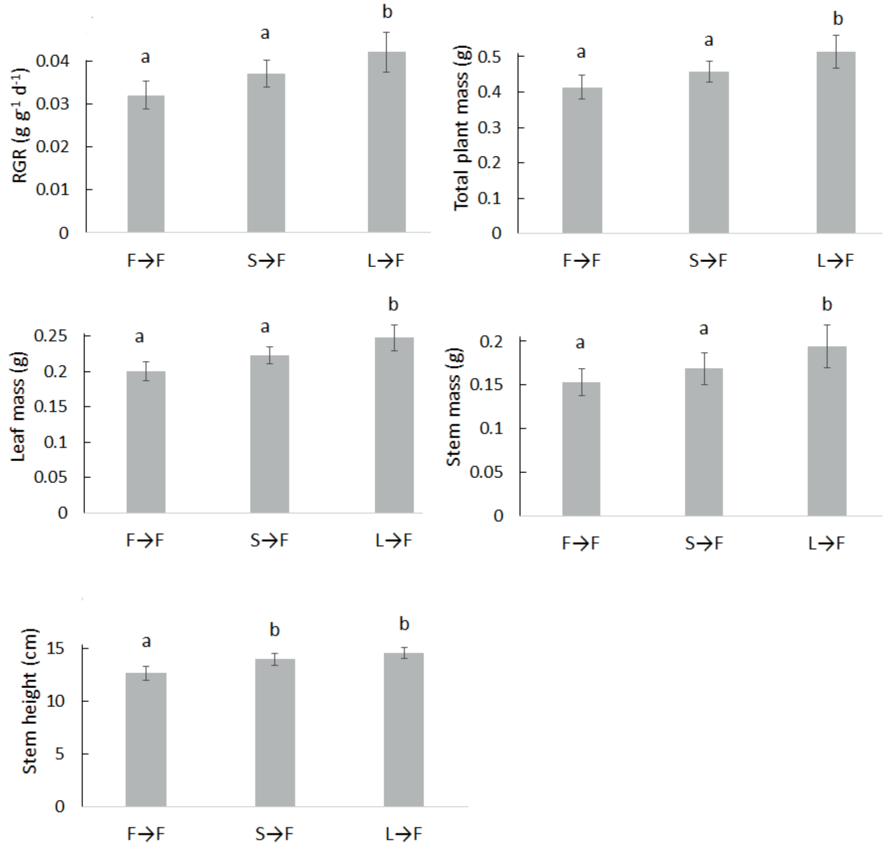

Figure 8. Mean plant trait values of cultivar Fairytale exposed to volatiles of Fairytale (F→F), Salome (S→F), and Luhkas (L→F), 25 days after sowing. Different letters indicate significant differences ($P < 0.05$).

Unexposed barley cultivars differed significantly in their volatile profiles (paper IV), a precondition of plant-plant communication; certain compound groups were more closely associated with some cultivars, such as terpenoids with Luhkas and alkanes with Fairytale. The volatile profiles of Salome and Luhkas and those of Fairytale and Anakin were compositionally similar (Fig. 9). Given the different growth pattern of Luhkas (fast) and Fairytale (slow), one could assume that the composition of their volatile profiles should also be dissimilar, which is in line with the analysed odour profiles (Fig. 9). The amount of individual components released (Appendix 8 in paper IV), does not seem to correlate with the individual growth patterns. However, it is most likely that plant communication is based on specific blends, rather than single compounds (Ueda et al., 2012). Nevertheless, the key point is that the exposed plants could recognize differences in the volatile profiles and responded accordingly to their neighbours.

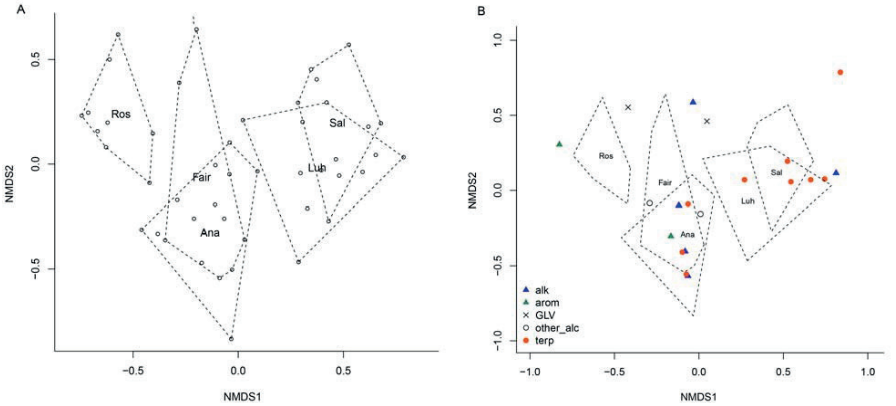

Figure 9. Nonmetric multidimensional scaling (NMDS) results illustrating compositional dissimilarity in volatile profiles between barley cultivars Anakin, Salome, Fairytale, Rosalina, and Luhkas. (A) Individual plants are shown with circles and group boundaries for cultivar types marked with dashed lines using the ordihull function in vegan (Oksanen et al., 2017). (B) Individual components, categorized into alkanes, aromatic compounds, green leaf volatiles, other alcohols, and terpenoids.

Further, if a volatile emitter plant of the tested cultivars had higher stem- or root related traits than the exposed Fairytale, Fairytale plants responded with increased growth of the same traits, as shown by analysis of pairwise correlations (Fig. 3 in paper II). Positive correlations were found for traits related to foraging for light and nutrients. There was no shortage of either light or nutrition during the experiment. Therefore, there is a basis for the assumption that the exposed Fairytale plants ’predict’ the likelihood of shading or nutrient shortage in the future (mediated by volatiles from putative competitors), and their growth responses can be seen as a preparation for future competition, anticipating future resource deficits. It has been shown that plants initiate phenotypic changes (shade avoidance) in response to changes in far-red/red light cues (Pierik and de Wit, 2014). Such shade avoidance can be initiated by low light reflected from nearby plants before any actual loss of photosynthetic light, i.e. the phenotypic changes of shade avoiding plants adapt to future conditions rather than the present (Novoplansky, 2016; Trewavas, 2017). Root growth has also been shown to assess future acquisition of resources (Takahashi et al., 2002). Roots of pea plants developed more roots in patches with an increasing level of nutrients although less rich in absolute terms, compared to patches with no nutrient increase (Shemesh et al., 2010). Thus, plants are not only able to perceive and respond to changes in their environment, but their response can be focused on both current and anticipated conditions.

With the same experimental setup as in our study, Ninkovic (2003) and Kegge et al. (2015) showed, that a barley cultivar that was exposed to volatiles from another cultivar allocated its biomass to other plant parts, and that this biomass allocation was dependent on (a) the *cultivar identity* and (b) the *light environment of the volatile emitter plant*. In our experiment, we also wanted to describe the emitter plants, so we analysed their *growth patterns*. Our study is the first to show that plants can gain information about the growth pattern of volatile emitting plants and respond with similar growth strategies. This contributes to a better understanding of volatile communication between plants and its impact on plant life. If it turns out at longer experiment durations, that the fitness of exposed plants is affected through higher yields, it could be of enormous value for plant breeders and farmers. Plant volatile emissions can be modified by plant breeding (Åhman et al., 2010) to create cultivars that can induce higher productivity in their plant neighbours, or by cultivars with different growth strategies that are grown together in fields to increase crop yields. Whether such volatile communication can be useful in agricultural settings will be discussed in the next two sections.

3.3 Phenotypic plasticity (paper III)

As the growth adaptations found in paper II are properties of individuals, it is necessary to scale from the individual plant to the level of communities in order to understand the indirect effects of a plant's adaptive capacity. Therefore, we also tested plant-plant interactions in field experiments with cultivars grown in mixtures and pure stands. The results show that plastic growth changes were also found when two cultivars were grown together in the field. Plants in cultivar mixtures developed slower early in the season, and grew faster later on (Fig. 10). Such slower development was also found for volatile exposed plants in the laboratory (paper II). The new environment (another neighbour) might be a stress factor resulting in slower development of plant growth. When this stress is permanent, the magnitude of the stress response decreases, plants habituate and can invest again in faster growth.

Significant differences in plant height of cultivars grown in mixtures and pure stands were found already 40 days after sowing, with differences of up to 3 cm

(Fig. 10 A). With a mean plant height of 8 cm and a row space of 12.5 cm, it would not be possible that the cultivars in mixtures had touched or shaded each other at this time. An interaction via volatiles on the other hand would have been possible. Such volatile interactions between plants have not been considered to affect plant behaviour under field conditions, but our results suggest they should be taken into account in future research.

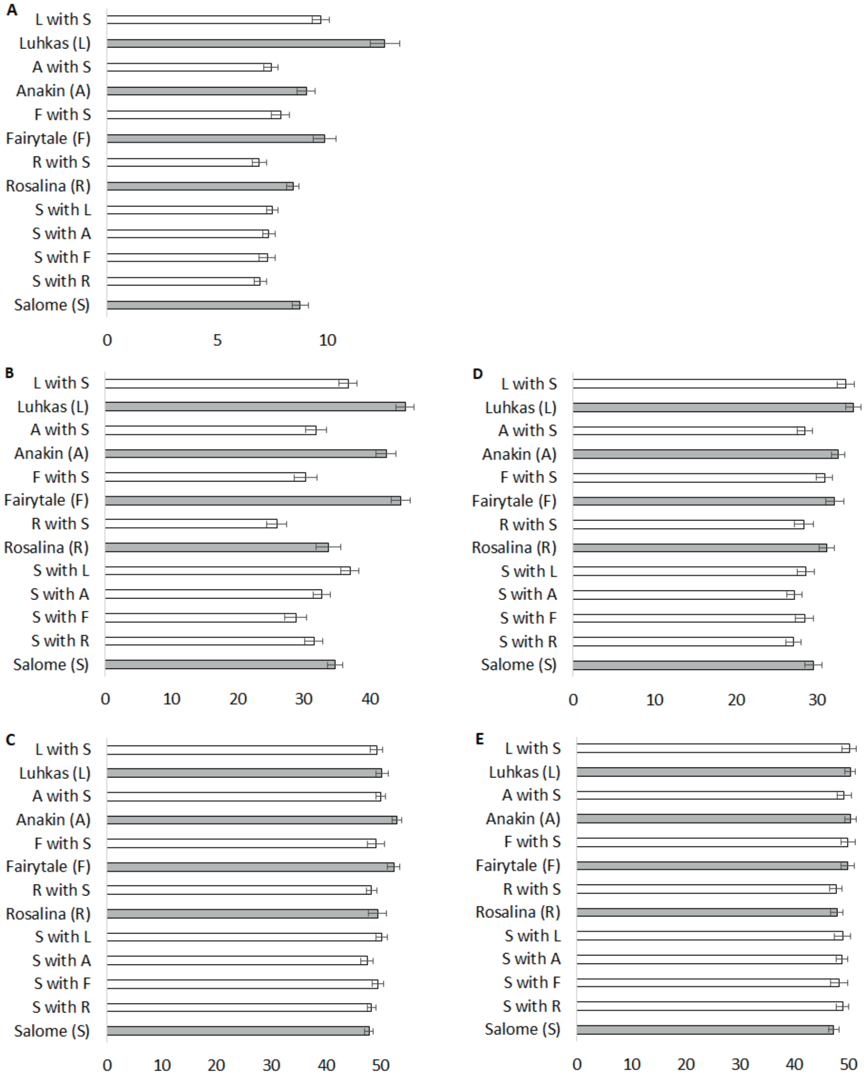

Figure 10. Plant height (cm) of cultivars grown in mixtures in all tested combinations (white bars) and in pure stands (grey bars), measured 40 (A), 52 (B) and 68 (C) days after sowing in 2015 and 47 (D) and 57 (E) days after sowing in 2016. Error bars indicate the sample standard errors.

Mixing generally promoted final biomass and grain production of cultivars (Table 2 in paper III) and this was dependent on the neighbour cultivar (Table 5 in paper III). The mixture Salome-Rosalina for example produced significant higher biomass and grain yield than any other cultivar combination (Table 5 in paper III). If this response could be predicted from data on plant performance of the component cultivars in pure stands, it would be an additive effect (Hughes et al., 2008). This is not the case, as the observed per plant biomass and kernel production of this mixture was 27% and 22% higher than their expected values. Thus, we have observed a non-additive response to mixing through interactions among coexisting cultivars, which could have been caused through differentiation of their niches or diverse patterns of resource use. In fact, Salome and Rosalina had a higher nitrogen accumulation efficiency (NAE) when grown together compared to other cultivar combinations (Table 5 in paper III). Further, the NAE did not became more similar when these two cultivars grew together, compared to the NAE difference between them when grown in pure stands (Table 6 in paper III). Thus, the NAE of Salome and Rosalina was higher and more dissimilar when they grew together compared to pure stands; they had a higher separation of their niches and thereby competed less with each other, which may have contributed to the increased biomass and grain production. If these non-additive responses to mixing through interactions among coexisting cultivars could have been caused by volatile interactions, we currently cannot answer, but this possibility is not excluded.

Such individual mixture responses rather than only the cultivar average, can greatly improve the predictive value of cultivar mixtures and help design guidelines for selecting cultivars to be mixed (Barot et al., 2017). Literature on the impact of crop genotypic diversity on productivity reports opposing outcomes; genotypic mixtures do not always give higher yields than their component pure stands (Reiss and Drinkwater, 2017; Borg et al., 2018). Most of these studies only assessed differences between cultivars grown in mixtures or single stands, without measuring plant responses to individual mixture components. Our results indicate that plant responses to the individual neighbour might be an important driver behind the variability in the mixing effects reported by previous studies.

The next section will discuss whether volatile communication could have affected plant interactions in the field experiments and what effect this had on aphid performance.

3.4 Aphid responses (paper IV)

Plant growth- and physiological changes in response to volatile signals can affect other organisms that use the plant as a host (Dicke, 1994; Ninkovic et al., 2013). Aphids are very sensitive to slight changes in their host plants, and their behaviour and abundance can be affected by any differences (Ninkovic et al., 2016). The exposure of barley plants to volatiles from another cultivar resulted in significantly reduced aphid-plant acceptance in six cultivar combinations (Table 2). The exposure effects were both receiver and emitter specific; Fairytale and Salome induced strong effects as emitters, whereas Anakin was a very responsive receiver. Plant acceptance is essential in the progress of an aphid infestation (Pettersson et al., 2007) and is correlated with subsequent aphid growth rate (Ninkovic et al., 2009; Dahlin & Ninkovic, 2013), which might affect aphid population development.

Table 2. The ratio of mean number of aphids that accepted plants treated with volatiles to the mean number of aphids that accepted control plants. Reduced acceptance gives a ratio of <1, while a ratio of 1 indicates no difference between treated and control plants. Significant differences in estimates are indicated by asterisk (*P < 0.05; **P < 0.01; ***P < 0.001).

			Emitter		
Receiver	Anakin	Fairytale	Luhkas	Rosalina	Salome
Anakin	x	0.85**	0.96	0.87*	0.80***
Fairytale	1.03	x	0.95	0.91	0.84**
Luhkas	0.94	1.04	x	0.95	0.87**
Rosalina	0.99	1.00	1.01	x	0.97
Salome	0.93	0.85**	1.12*	0.96	x

Aphid populations developed differently in cultivar mixtures, possibly induced by volatile interactions between cultivars, making them less susceptible to aphids. Cultivars Salome and Fairytale interacted with each other with a strong effect (31% and 22% reduction respectively) on aphid population development. A limited reduction (13%) in aphid population was also observed on Salome grown with Anakin, while the other cultivars showed a similar population development on monocultures and mixtures. This suggests that aphid responses to one cultivar in a mixture are neighbour-specific. A reduction, which could be of practical relevance to agriculture, was only observed in the Salome-Fairytale mixture, where aphid-plant acceptance in the laboratory and population growth in the field were reduced on both mixture components (Table 2 and Fig. 1 in paper IV). This is in line with a simulation study on aphid population development in cereals (Wiktelius and Pettersson, 1985), which estimated that a 20% reduction in aphid population growth during plant acceptance leads to 40% reduction in peak aphid numbers.

The results from the field experiment show that peak aphid populations were generally lower in all four cultivar mixtures than in monocultures (Fig. 11). Even though none of the used cultivars are resistant to aphids, it seems that they differ in their susceptibility, because aphid populations varied between cultivars grown in pure stands, with higher populations in Salome, Anakin and Luhkas than the other cultivars (Table 2 in paper IV). However, a cultivar combination with low susceptibility (Salome-Anakin) had the same population size as a combination with high and low susceptibility (Salome-Rosalina). Therefore, we suggest that cultivar identity is crucial for pest suppression in mixtures, but the driving mechanism is not based on variation in susceptibility between cultivars, which is proposed to occur via the dilution effect where a resistant component reduces pest spread to the susceptible component (Mundt, 2002).

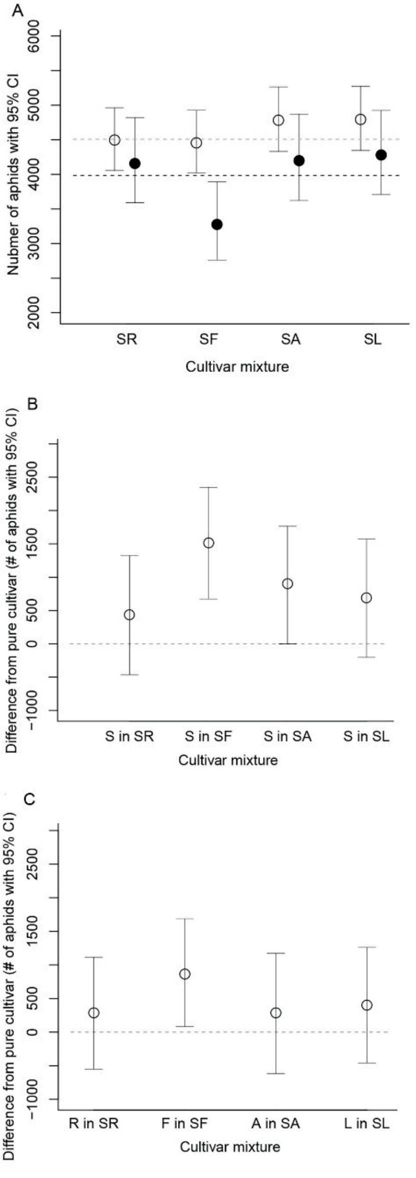

Figure 11. Expected and observed aphid population size per plot in mixtures and differences between individual barley cultivars grown in pure stands and mixtures, estimated by the population model (mean with 95% CI). (A) Expected (empty symbols) and observed (black symbols) aphid population sizes in cultivar mixtures with the average estimated population size in pure stands (grey line) and mixtures (black line); (B) Salome (S) grown with other cultivars (A, F, L, R) grown with S. Positive differences indicate higher population sizes in pure stands.

No differences in aphid immigration between cultivar mixtures and monocultures were found when we analysed the catches of winged aphids in yellow water traps in the field experiments. We found no relationship between predator abundance and aphid abundance (Appendix 6 in paper IV). Therefore, it is likely that the lower population size in some of the mixtures is mediated through plant interactions (bottom-up) and not by higher abundance of natural enemies (top-down), as shown in other studies (Johnson et al., 2006; Grettenberger and Tooker, 2017).

The same cultivar combinations that were less accepted by aphids after exposure in the laboratory were also less attacked in field trials. This, together with the findings from paper III, shows that plant interactions that will contribute to plant fitness (through higher productivity or reduced aphid performance) (a) must take place early in a plant's development, (b) are (therefore) mediated by volatile compounds, and (c) depend on both of the mixed cultivars 'fitting together'. By 'fitting together' we mean, with regard to effects on the impairment of aphids, that both emitters and receivers mutually respond to each other's volatile cues. With regard to higher productivity, we do not know why some cultivars 'fit together' better than others. Some cultivars might have a greater plasticity to adapt to each other. Others might not need to adapt to neighbours due to larger trait differences. Some plant interactions might not be strong enough to induce desired effects, or some plants might not respond to volatile cues, because their neighbours were not considered as potential competitors. Nevertheless, we have demonstrated the phenomenon of plant neighbour responses via volatiles and their effects on the plants themselves, and on aphid performance. We have tested only very few cultivar combinations; more data is needed to predict complementary interactions among cultivars to facilitate the choice of cultivars to be mixed. This will open so far unexplored potential to optimize cultivar mixtures for improved crop production combined with increased resistance against insect pests.

4 Conclusions and future directions

This thesis addressed the question of how plant-plant communication affects plant and aphid responses, and whether increased botanical diversity in agricultural fields via cultivar mixtures affects crop productivity and pest suppression.

We demonstrated:

Plant volatile communication is a general event not limited to damaged plants and is more a phenomenon of volatile eavesdropping rather than signalling.

Plant volatiles carry information about the growth development of their emitters.

Volatile exposed plants can perceive this information and respond with similar growth strategies.

Volatile induced plant adaptations to neighbour growth rates might anticipate future resource deficits and can be seen as a preparation for future competition.

Plant volatile communication and induced growth changes take place under both laboratory and field conditions.

Aphid-plant acceptance of volatile exposed plants was reduced in some cultivar combinations, and this response was receiver and emitter specific.

Growth development of cultivars grown in mixtures initially slows, but accelerates after acclimation.

Plant interactions that will lead to increased plant fitness (through increased productivity or pest suppression) must take place early in a plant's development.

Cultivar mixtures generally had higher productivity and pest suppression than cultivars grown in pure stands.

This mixing outcome was caused neither through additive- nor dilution effects, nor due to aphid colonization or natural enemy abundance.

Mixing effects on productivity and pest abundance were neighbour specific and most pronounced in a few cultivar combinations.

Such individual neighbour responses can explain the variability in reported mixture effects from studies evaluating cultivar mixtures *per se*.

Research on plant responses to volatile cues has demonstrated the capacity of plants to modify their strategies to meet a diversity of ecological challenges. This thesis contributes to this research by showing that plants can perceive information about growth strategies of their neighbours through volatiles, and are able to respond to such patterns with appropriate growth responses. Plants respond to their neighbour's identity with both adaptive and maladaptive growth responses affecting plant productivity and pest suppression. Plant adaptation to the local environment has evolved through survival of the most adaptive genotypes in the wild. We have shown that this adaptive ability is retained in some cultivars even after hundreds of years of crop breeding.

Future research taking the same approach as in this thesis should:

- conduct more field experiments preferably in different years. Field experiments show how the tested organisms are adapted to the prevailing abiotic conditions; such results are more applicable than results from artificial settings.
- continue with laboratory experiments on plant growth adaptation to examine whether volatile exposed plants responded to the cultivar identity or the size of plants.
- test cultivar responses to specific compounds or blends of volatiles that potentially mediate plant growth responses, to identify cultivars that produce higher yields/reduce pest damage when grown together.
- analyse whether there is a correlation between changed plant traits and aphid population development observed in field experiments.

Future research taking a new approach might:

- assess aphid behavioural responses to volatile exposed plants in olfactory and feeding experiments. Evaluation of olfactometer and EPG (electrical penetration graph) data can give indirect information about possible changes in the volatile profiles or host plant quality of exposed plants (Dahlin et al., 2015; Trębicki et al., 2016).
- analyse the phloem sugar composition of plants exposed to volatiles from another cultivar, because aphids can be negatively affected by an elevated C/N ratio in the phloem (Karley et al., 2002).
- evaluate the gut content of aphid natural enemies to detect trophic links between predators and *R. padi.* Pitfall trap catches from field experiments only provide information on the activity density of natural enemies, but a molecular gut content analysis can reveal if the captured predators have contributed to the biological control of these aphids (Szendrei et al., 2010). This method also provides information about intraguild predation or cannibalism (predators feed on each other), which might disrupt biological control of aphids (Szendrei et al., 2010).
- investigate gene expression of plants in response to volatile exposure of neighbouring plants, because defence genes might be activated and could have affected aphid-plant acceptance (Markovic et al., 2019).

When tailoring efficient, stable and predictable cultivar mixtures, one should not choose cultivars by their characteristics when grown in pure stands. Plant traits are highly plastic and one genotype can express different phenotypes depending on its plant neighbour. Cultivars should instead be selected based on how they interact with each other. So far, research has not elaborated a framework that would allow selecting the best cultivar combinations. Future research could make cultivar mixtures a more reliable tool for productive and sustainable agricultural systems.

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Plant communication in cultivar mixtures: From plant to aphid responses

In this thesis, the chemical interaction between barley cultivars has been studied. The studies show that growing two cultivars in a field instead of a single cultivar can reduce infestation by insect pests and increase yield. Cultivar mixtures can thus reduce the need for both chemical pesticides and fertilizers, making an important contribution to agricultural sustainability.

Previous research on how cultivar mixtures affect pests and plant productivity has not always shown positive effects on insect pests or yields. Therefore, we need more knowledge about the processes behind the responses of pests and plants in cultivar mixtures to explain why some studies find positive effects whereas others do not.

Volatile communication between plants is one possible explanation. Plants constantly emit volatile chemical substances, and other plants nearby can perceive these chemical signals. Most studies on volatile communication in plants have focussed on what happens when a plant is attacked by pests. Nearby plants can perceive damage-induced volatiles from their neighbours and prepare their own defences before they are attacked themselves. Our studies show that plants can also respond to chemical signals from undamaged neighbours.

In laboratory experiments, we showed that barley plants could, via volatile substances, perceive how fast their neighbours grew and that they could adapt their own growth accordingly. This can be seen as a preparation for future competition.

In field experiments, cultivars grown together in mixtures responded differently depending on which other cultivar was nearby. Some cultivars adapted their growth to match their neighbours whereas others did not. Consequently, some cultivars in mixtures became more efficient in collecting nitrogen and producing higher yield.

When certain cultivars were grown together, aphid attacks also decreased. Other factors such as the immigration of aphids or the number of their natural enemies did not contribute to this effect; it depended only on the cultivars included in the mixture.

To test whether this effect was due to volatile substances, we exposed plants of one cultivar to volatiles from another cultivar in specially designed two-chamber exposure cages. After five days of exposure, some cultivar combinations were significantly less attractive to aphids, and these same combinations were less affected by aphid attacks in the field.

Overall, our findings support the hypothesis that response to volatiles from neighbouring plants made the plants less attractive to aphids in the field and producing more yield. The response of both plants and aphids to a cultivar in a mixture was neighbour-specific and most pronounced in certain cultivar mixtures. Such individual responses to neighbours mediated by volatiles can explain the varying effects of cultivar mixtures reported from previous research.

The results of this thesis provide a better understanding of volatile communication in plants, adding a new dimension to plant behaviour and processes in plant communities. It also lays the foundation for a better understanding of the underlying mechanisms in interactions between cultivars, which can lead to the development of sustainable plant protection against insect pests and higher productivity in cultivar mixtures in future agriculture.

Växtkommunikation i sortblandningar: Från växt till bladlusreaktioner

I denna avhandling har det kemiska samspelet mellan kornsorter studerats. Studierna visar att man kan minska angrepp av skadeinsekter och öka avkastningen när man odlar två sorter på en åker istället för en enda sort. Sortblandningar kan därmed minska behovet av både kemiska bekämpningsmedel och gödsel, vilket gör ett viktigt bidrag till hållbarheten inom jordbruket.

Tidigare forskning om hur sortblandningar påverkar skadegörare och växtproduktivitet har inte alltid visat positiva effekter på skadeinsekter eller skörd. Därför behöver vi mer kunskap om de processer som påverkar skadegörare och växter i sortblandningar för att förklara varför vissa studier hittade positiva effekter, medan andra inte gjorde det.

Kommunikation mellan växter kan vara en möjlig förklaring. Växter utsöndrar ständig flyktiga kemiska ämnen och andra växter i närheten kan snappa upp dessa kemiska signaler. Hittills har de flesta av studierna om kommunikationen mellan växter fokuserat på vad som händer när en växt blir angripen av skadegörare. Närliggande växter kan snappa upp skada-inducerad flyktiga ämnen från deras grannar och förbereda sitt eget försvar innan de själva blir angripna. Våra studier visar att växter också kan reagera på kemiska signaler från oskadade grannar.

I laboratorieförsök visade vi att kornplantor, via flyktiga ämnen, kunde få information om hur snabbt deras grannar växte och att de kunde anpassa sin egen tillväxt därefter. Detta kan ses som en förberedelse för framtida konkurrens.

I fältförsök reagerade sorter som odlades tillsammans i blandningar olika beroende på den andra sort de odlades med. Vissa sorter anpassade sin tillväxt för att matcha sina grannar medan andra inte gjorde det. Följaktligen samlade vissa sorter i blandningar på sig kväve mer effektivt och gav högre avkastning.

När vissa kornsorter odlades tillsammans minskade också angrepp av bladlöss. Detta berodde på vilka sorter som ingick i blandningen, och inte på andra faktorer såsom inflygning av bladlöss eller antalet naturliga fiender.

För att testa om denna effekt orsakades av flyktiga ämnen, utsatte vi plantor av en kornsort för luftburna flyktiga ämnen från en annan sort i specialdesignade tvåkammar exponeringsburar. Plantorna var instängda i varsin bur där flyktiga ämnen från en sort fördes till plantor av en annan sort. Efter fem dagars exponering var några sortkombinationer betydligt mindre attraktiva för bladlöss. Samma sortkombinationer drabbades mindre av bladlusangrepp i våra fältförsök.

Sammantaget stödjer experimenten teorin att det var reaktionen på flyktiga ämnen från angränsande växter som gjorde plantorna mindre attraktiva för bladlöss i fältet och att de gav högre avkastning. Respons av både växternas och bladlössens respons på en sort i en blandning var grannspecifik och mest uttalad i vissa sortkombinationer. Sådana individuella reaktioner till grannar som förmedlats av flyktiga ämnen kan förklara de varierande effekterna av sortblandningar som rapporterats i tidigare forskning.

Resultaten av denna avhandling ger bättre förståelse av kommunikation mellan växter, vilket lägger en ny dimension till växtbeteende och processer i växtsamhällen. Den lägger också grunden för en bättre förståelse av underliggande mekanismer i samspel mellan sorter, vilket kan leda till utvecklingen av hållbart växtskydd mot skadegörare och högre produktivitet i sortblandningar i framtidens jordbruk.

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Figure 12. Even insectivorous plants have a heart. By Catfisheye/CC BY 4.0.

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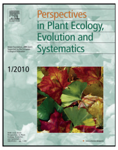

Review

Decoding neighbour volatiles in preparation for future competition and implications for tritrophic interactions

Velemir Ninkovic[a,*], Dimitrije Markovic[a,b], Iris Dahlin[a]

[a] Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Faculty of Agriculture, University of Banja Luka, Banja Luka, Bosnia and Herzegovina

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ABSTRACT

Plant volatile signals can provide important information about the physiological status and genetic identity of the emitter, and nearby plants can use this information to detect competitive neighbours. The novelty of these signals is that plants eavesdropping to volatiles of undamaged neighbours respond with typical competition responses, even before competition takes place, initiating specific growth responses that can increase their competitive capacity. This preparing for future competition mechanism affects the behaviour and abundance of herbivore pests and their natural enemies. Previously, such responses were only known to occur in response to volatiles released by damaged plants. However, volatile interactions occur only in specific combination of species/genotypes, indicating that plants use volatile signals in the detection and adaption only to substantial competitive neighbours.

Contents

1. Plant volatile signals

From its first moment, a growing plant is exposed to various challenges affecting its survival and the plant can respond to this in different ways. Growth condition at the site sets a frame for plant resources to respond to these changes. By spending a lifetime rooted to the same place, as a consequence of their specific nature, neighbouring plants constantly share the same available resources. Thus, coexistence with other plants is permanent and the most important challenge that individual plants face during their life cycle. In order to prepare for competition with nearby plants and possible upcoming threats, plants monitor and detect reliable signals, to which they respond with great sensitivity and discrimination (Ballarè and Casal, 2000; Clark et al., 2001; Trewavas, 2005). In order for a plant to survive, it must detect the presence of competing individuals, both of the same species (conspecific) and different species (heterospecific), and then adapt appropriately (Hutchings and Dekroon, 1994; Callaway and Aschehoug, 2000; Fridley et al., 2007; Murphy and Dudley, 2009; Ruberti et al., 2012). The consequent signalling that plants perceive forces them to distinguish between crucial signals predicting competitive neighbours from insignificant ones not crucial for their own fitness. Plants

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* Corresponding author at: Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Box 7043, SE-750 07 Uppsala, Sweden.
E-mail address: velemir.ninkovic@slu.se (V. Ninkovic).

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respond to competitors through physiological and morphological changes that increase their fitness (Callaway et al., 2003; Crutsinger et al., 2006; Violle et al., 2009). They have developed strategies such as competition, confrontation and tolerance (Novoplansky, 2009) to outgrow (Franklin, 2008), suppress (Inderjit et al., 2011) or tolerate (Valladares and Niinemets, 2008) proximate neighbours.

Plants detect neighbouring plants through different kinds of signals, such as quality of light (Izaguirre et al., 2006; Franklin, 2008; Keuskamp et al., 2010), acoustic (Gagliano et al., 2012; Appel and Cocroft, 2014), root exudates (Biedrzycki et al., 2010), root emitted volatile organic compounds (Delory et al., 2016), airborne volatile organic compounds (Ninkovic et al., 2013), floral volatiles (Caruso and Parachnowitsch, 2016) and touch (Braam, 2005; Markovic et al., 2014). Among the crucial signals are airborne volatile signals, which are constantly released by plants into their surroundings. The adaptive strategy of the plants exposed to volatiles depends strongly on the emitter's identity (Ninkovic, 2003; Kellner et al., 2010) and its physiological status (Braam, 2005). Physiological changes in plants responding to volatile signals can cause changes, such as different volatile profiles, which can then be perceived by other plants and organisms (Ninkovic et al., 2013; Dahlin et al., 2015). This paper aims to review the present knowledge on airborne volatile-mediated interactions between plants and the implications of these interactions on different trophic levels. We also identify some research areas that call for increased attention.

2. Volatiles as signals in detection of competitive neighbours

Volatile organic compounds (VOCs) can offer important informative value about the physiological stage of each individual in plant communities. The production and emission of VOCs is developmentally regulated, increasing during the early stages of the development when leaves are young and decreasing after maturity (Dudareva et al., 2000). The way in which plants respond to these volatile stimuli depends heavily on the significance of perceived information and neighbour identity, which can be highly related to the age of the receiver. Thus, younger plants are more responsive to future competition than older ones (Novoplansky et al., 1990). Since the emitter plant releases volatile signals constantly in its environment, it can be exploited by nearby plants as a cue for competitive neighbours, thereby initiating growth responses that increase the competitive power of eavesdropping plants (Dicke et al., 2003; Heil and Karban, 2010). The genetic identity of neighbours can have a significant impact on the receiver's growth and development, since the plants share the same available resources but may have different needs. The capacity of an individual plant to recognise nearby kin or strangers and respond differently to their presence represents an important trait that helps plants adjust their competitive ability to a specific neighbour (Fridley et al., 2007; Murphy and Dudley, 2009).

Volatile emissions from undamaged neighbouring plants can be important signals in the process of plant adaption to the presence of potential competitors. For example, Ninkovic (2003) tested two barley varieties that were exposed to each other in laboratory experiments where all other types of interactions were prevented except via volatiles. Plants of the barley variety Kara that had previously been exposed to VOCs of variety Alva allocated more biomass to their roots than unexposed plants or Kara exposed to VOCs of other Kara plants. An increased root biomass in young receiver plants may contribute to their fitness by boosting their capacity for below-ground competition through root proliferation into nutrient-rich patches. A decreased red:far-red light act as the earliest neighbour-detection signal in competition for light (e.g., Dicke and Baldwin, 2010; Pierik and de Wit, 2014) which induces elongation and affects the VOCs' emission rate of exposed plants (Kegge et al., 2013). In another experiment, the emitting Alva plants grown in low red:far-red conditions showed typical shade avoidance, increasing in biomass allocation to shoots and changing emission of their volatile blend (Kegge et al., 2015). Such altered volatile emission of Alva induced a typical shade avoidance response of exposed Kara plants that accumulated more resources into shoot- and leaf-biomass than to roots. These examples show that VOCs acts as detecting signals that have important informative value about the physiological status of neighbouring plants, which can induce responses in receiving plants to prepare for future competition. The extraordinary novelty of plants' ability to use volatile cues to predict the existence of forthcoming competitive neighbours is reflected in the response that occurs even before competition takes place. This preparing for future competition mechanism also operates between undamaged neighbours of different species: potato plants that were previously exposed to volatiles from onion plants changed their volatile profile by releasing considerably greater quantities of two terpenoids (Ninkovic et al., 2013). Such responses were previously only known to occur in response to volatiles released by damaged plants (Dicke and Baldwin, 2010; Karban et al., 2014). Thus, VOCs carry information about whether neighbouring plants are under attack, but also about the emitter plants themselves, which enables them to make specific preparations for future competition.

The above examples show that VOCs (a) act as neighbour detection signals, (b) mediate inter- and intraspecific plant interactions, (c) have important informative value about neighbouring plants, and (d) induce responses in receiving plants that prepare for future competition. However, there is a need for further studies to provide knowledge about the underlying mechanisms that are responsible for plants' ability to adapt to competitive neighbours by responding to their volatiles. Interactions between plants are very complex and may have significant ecological implications. The fact that the behaviour of insects can be affected gives this phenomenon even wider ecological significance.

3. VOCs induced responses and tritrophic interactions

Volatile interactions between undamaged plants induce changes in receiving plants with the potential to influence organisms at higher trophic levels (Fig. 1A and Table 1) (Glinwood et al., 2011; Ninkovic et al., 2013). The term 'allelobiosis' has been introduced to describe this process and its effects on receiving plants and at higher trophic levels (Pettersson et al., 2003; Ninkovic et al., 2006). In natural habitats, the leaves of birch *Betula* spp. adsorb and then re-release specific herbivore repelling volatiles produced by *Rhododendron tomentosum* Harmaja, reducing their attractiveness to herbivorous insects (Himanen et al., 2010). Broccoli also showed the same ability to adsorb and re-release *R. tomentosum* volatiles, becoming less susceptible to *Plutella xylostella* (L.) oviposition and less favoured and damaged by their larvae (Himanen et al., 2015). The changed volatile emission of onion-exposed potato plants in the above mentioned example resulted in the avoidance of both winged and wingless *Myzus persicae* (Sulzer) morphs (Ninkovic et al., 2013; Dahlin et al., 2015), indicating that active response to volatiles from neighbouring plants may even have effects on herbivorous insects. However, this only occurs in specific combinations of plant species. Thus, volatile chemical interactions between different weed species and barley only affected aphid plant acceptance after exposure of two weed species, indicating that these types of interactions are dependent on the plant species involved (Glinwood et al., 2004; Ninkovic et al., 2009; Dahlin and Ninkovic, 2013).

It has been hypothesised that diversified crops cause a reduction in the abundance of herbivorous insects (Norris and Kogan, 2005).

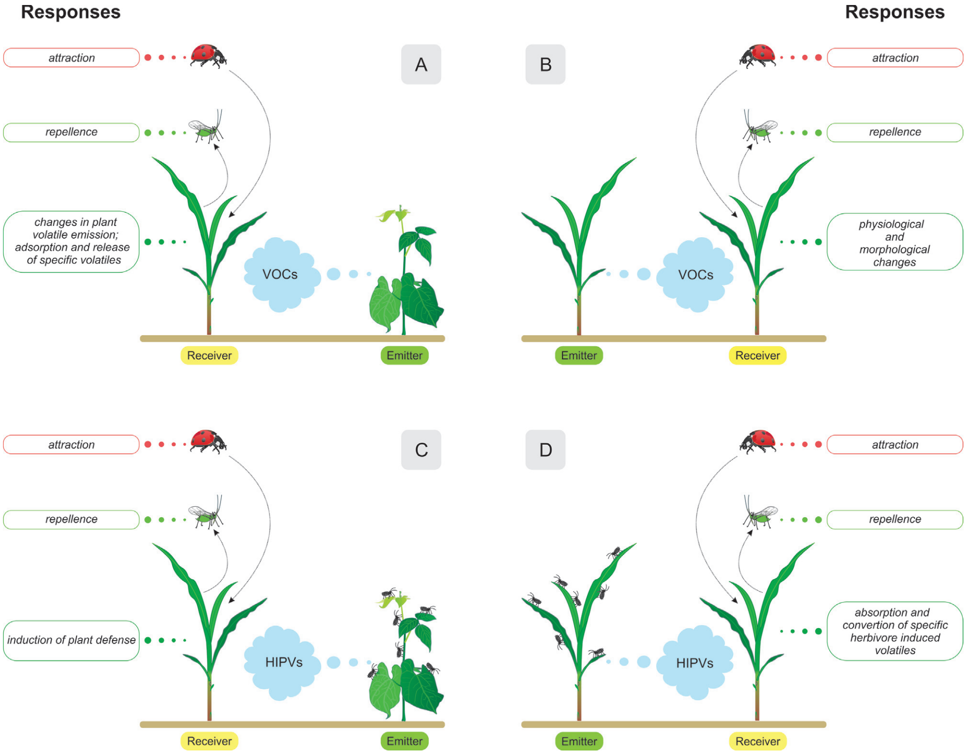

Fig. 1. Volatiles released from nearby undamaged (A) heterospecific and (B) conspecific competitor induce adaptive responses in receiving plant with further implications on other trophic levels. (C) Changed volatile emission after damage (herbivore or mechanical force) of heterospecific or (D) conspecific competitors induce adaptive responses in receiving plants with further implications on other trophic levels.

However, some studies have indicated that diversification had no effect, or increased herbivore densities. In a review of 150 studies on the effects of diverse agro ecosystems on insect herbivores, Risch, Andow and Altieri (Risch et al., 1983) found that 53 per cent of the herbivore species were less abundant in diverse systems, 18 per cent increased, 20 per cent showed a varied response and 8 per cent did not differ between the systems. The variable effect of increased botanical diversity on the occurrence of herbivores could be due to differences in the adaptability of plants to respond to neighbours. The fact that not all plants responded to volatile signals from their specific neighbours suggests that plants may not respond to insignificant signals or may not consider their neighbours as potential competitors. Thus, the ability of plants to adapt to a specific neighbour is dynamic, which can have different outcomes on specific plant–insect interactions (Dahlin and Ninkovic, 2013). Neighbouring plants that are not considered competitors still may have a beneficial role to focal plants due to the processes of associational resistance (Barbosa et al., 2009) which may make plants less exposed to pest attack (Marquis et al., 2002; Himanen et al., 2015).

However, VOCs have been shown to even induce responses in different varieties/genotypes of the same plant species that affect plant defence against herbivores (Fig. 1B). Changes in the growth of receivers and biomass allocation patterns in barley, after exposure to another variety (Ninkovic, 2003), indicate that certain physiological changes within plants could have further implications on herbivores. Pettersson et al. (1999) were the first to show that volatile communication between different barley varieties may reduce the acceptability of exposed plants for *Rhopalosiphum padi* L. Certain combinations of barley genotypes, followed by volatile exposure, significantly reduced aphid acceptance, both in laboratory and field experiments (Ninkovic et al., 2002). As a consequence of the selection process, some barley varieties became good signal emitters while other varieties became better receivers. In general, older barley genotypes displayed a greater tendency to respond to volatile exposure, whereas more recent ones are more likely to be inducers (Kellner et al., 2010). Genotypes that had shown a reduced aphid acceptance also responded to volatile exposure from a particular different genotype with lower aphid growth (Ninkovic and Åhman, 2009). Studies have shown that interaction between plants in diverse wheat variety mixtures reduces *R. padi* performance, affecting mother aphid size that decreased offspring production and lower aphid population (Shoffner and Tooker, 2013; Grettenberger and Tooker, 2016). The empirical results of these studies support the notion that volatile communication represents an effective and rapid means of signalling among plants providing information of the same or different individuals as the emitter.

In plant population, individuals react differently to cues of surrounding plants, which may benefit responders by increasing the

Table 1
Survey of the examples on volatile communication between undamaged plants of the same and different species with the specific effects on tritrophic interactions due to induced responses of the receiving plants.

Author(s)	Emitter plant	Receiver	Induced effects
a) Between different plant species			
Glinwood et al. (2004)	Certain weed species	Barley	Reduced aphid acceptance
Ninkovic et al. (2009)	Certain weed species	Barley	Reduced aphid acceptance
Dahlin and Ninkovic (2013)	Certain weed species	Barley	Reduced aphid population development
Ninkovic et al. (2013)	Onion	Potato	Changed volatile profile, reduced aphid host plant acceptance and aphid immigration rate
Dahlin et al. (2015)	Onion	Potato	Reduced aphid plant acceptance
Himanen et al. (2010)	Rhododendron tomentosum	Betula pendula	Adsorption and re-release of herbivore repelling volatiles
Himanen et al. (2015)	*R. tomentosum*	Brassica oleracea	Adsorption and re-release of specific volatiles, reduced oviposition and larval feeding
Ninkovic and Pettersson (2003)	Couch grass/thistle	Barley	Increased attractiveness of ladybirds
Vucetic et al. (2014)	Onion	Potato	Changed olfactory responses of aphids, increased attractiveness of ladybirds
b) Between varieties of the same plant species			
Ninkovic (2003)	Barley	Barley	Biomass allocation to roots
Kegge et al. (2015)	Barley in reduced light	Barley	Biomass allocation to shoots
Pettersson et al. (1999)	Barley	Barley	Reduced aphid acceptance and growth rate in certain varieties combinations
Kellner et al. (2010)	Barley	Barley	
Ninkovic and Åhman (2009)	Barley	Barley	
Ninkovic et al. (2002)	Barley	Barley	Reduced aphid acceptance in certain varieties combinations
Shoffner and Tooker (2013)	Wheat	Wheat	Reduced aphid growth rate
Grettenberger and Tooker (2016)	Wheat	Wheat	Reduced aphid offspring production
Ninkovic et al. (2011)	Barley	Barley	Increased attractiveness for ladybirds

inclusive fitness of close neighbours. There is a clear benefit in the VOCs' detection and response to the presence of the "right" neighbour (specific genotype), as it may affect insect behaviour. The above mentioned examples confirm the benefit for signal receivers and strengthen the hypothesis that an increasing genotypic diversity in crop fields could greatly improve insect pest management (Cantelo and Sanford, 1984; Power, 1991; Ninkovic et al., 2002; Tooker and Frank, 2012; Grettenberger and Tooker, 2015). Interactions between plants are context dependent and influenced not only by species or genotype but also by the environment and the physiological state of the plants (Andow, 1991; Barbosa et al., 2009; Barton and Koricheva, 2010).

4. Herbivore predator responses to volatile interactions between undamaged plants

Interactions between undamaged plant species can lead to the alternation of habitat and prey searching behaviour of predatory insects, even when prey were not present (Fig. 1A) (Price et al., 1980; Bottrell et al., 1998; Ninkovic and Pettersson, 2003; Glinwood et al., 2009; Ninkovic et al., 2011). Thus, ladybird occurrence was significantly higher in patches containing either couch grass *Elytrigia repens* (L.) Desv. ex Nevski or thistles *Cirsium arvense* (L.) Scop. then in weedless patches in a barley field (Ninkovic and Pettersson, 2003). Subsequent laboratory studies showed that it was not the VOCs of the weeds by themselves that attracted the ladybirds; instead, ladybirds were more attracted to the VOCs of barley plants that were previously exposed to VOCs from *C. arvense* than to that of unexposed barley (Ninkovic and Pettersson, 2003). These findings are in line with another study showing that ladybirds were significantly more attracted to onion-exposed potato that resulted in an increased emission of two terpenoids than unexposed potatoes (Vucetic et al., 2014). These findings suggest that changed VOCs induced by volatile communication between plants can affect attraction of predators, which can be an underlying mechanism that contributes to an increased abundance of natural enemies in botanically diverse fields (Vucetic et al., 2014).

Effects of volatile communication between genotypes of the same plant species on the third trophic level have also been reported (Fig. 1B) (Johnson, 2008; Glinwood et al., 2009; Ninkovic et al., 2011). Significantly more ladybirds were found in plots sown with two different barley varieties than in pure plots of either variety alone (Ninkovic et al., 2011). Supporting laboratory studies showed that ladybirds were attracted to VOCs of one variety exposed to another and also to the combined VOCs of two different varieties (Glinwood et al., 2009; Ninkovic et al., 2011). Theory suggests that increased plant species diversity cause a reduction in pest abundance due to an increased number of natural enemies (Andow, 1991; Haddad et al., 2009; Randlkofer et al., 2010). The question remains as to whether decreased pest abundance is caused by higher numbers of their natural enemies or by associational resistance of neighbouring plants that decreases the amount of damage to crop plants (Barbosa et al., 2009; Dahlin and Ninkovic, 2013).

5. Plant volatiles carry information about upcoming threats

Plants' volatiles can also carry information about potential upcoming threats from their surrounding neighbours. Herbivorous insects or mechanical damage rapidly initiate the assaulted plants to substantially change their volatile profile and release herbivore-induced plant volatiles (HIPVs) (Mithöfer et al., 2005; Wasternack et al., 2006; D'Auria et al., 2007; Mumm and Dicke, 2010) that are not typical for undamaged plants (Dicke, 1999; Hare, 2011). These HIPVs have important informative value for undamaged neighbours (Karban and Maron, 2002; Arimura et al., 2010), which helps them predict impending herbivore attack and induce plant defence responses, which make plants less attractive and suitable hosts for herbivores (Fig. 1C) (Heil and Kost, 2006; Baldwin et al., 2006; Karban et al., 2010; Pearse et al., 2013). In such situations, neighbouring unattacked plants may have a huge advantage compared to the signal emitter, which requires resources for defence that would otherwise be used in competition for above- and below-ground resources with undamaged neighbours. However, HIPVs released from a damaged plant also have an important informative role for the emitter itself as within-plant signals that aim to inform other organs of the same plant about the threat. The primary function of HIPVs released after tissue damages is to transmit signals within the same plant (Heil and Ton, 2008) but not to inform neighbours. This is of particular importance for plants, as the vascular signal transport is much slower than for volatile signalling (Orians, 2005).

Close relatives have more similar chemotypes, confirming that volatile signals from close damaged kin provide more reliable information than those obtained from strangers (Karban et al., 2014). Such individuals of the same chemotype exchange signals more effectively and were significantly less herbivore damaged than individuals of different chemotypes. A recent study showed that tomato plants absorbed (*Z*)-3-hexanol emitted by herbivore attacked conspecific neighbours and converted to (*Z*)-3-hexenylvicianoside that is effective in suppressing growth and survival of cutworms (Sugimoto et al., 2014). Also, maize plants infested by *Mythimna separata* (Walker) released a specific blend of volatiles that induce defence responses in conspecific neighbouring plants, reducing larval development immediately after exposure or up to five days later (Ali et al., 2013). The ratio between specific compounds and their concentration is crucial for receiving plants in preparation for upcoming threats. Wounded Pyrethrum plants, *Tanacetum cinerariifolium* (Trevir.) Sch. Bip. increased the emission of several terpenoids, which were only effective in the biosynthesis of pyrethrin in neighbouring undamaged plants when all of the five components were included in the blend (Ueda et al., 2012). Many of the inducible and highly reactive HIPVs were shown to have a limited life-time in the atmosphere, ranging from a couple of minutes up to 24 h (Yuan et al., 2009). A greater degree of resistance in receiving plants against herbivores is related to a longer exposure period and a higher accumulation of volatile compounds from infested plants (Choh et al., 2004).

It has been demonstrated that *Trifolium pratense* L. grown together with conspecifics significantly reduced the emission of total and herbivore induced volatiles compared to *T. pratense* grown together with *Dactylus glomerata* L. or growing alone (Kigathi et al., 2013). Such a response of *T. pratense* to the presence of conspecifics was attributed to a reduced possibility of attack by specialist herbivores and minimised eavesdropping of herbivore attack information by neighbours (Fig. 1D). Considering the fact that different plant species emit specific HIPVs blends and grow at different distances from each other, it is reasonable to state that the defence induction in receiving plants is highly correlated to exposure time, emitter relatedness and the reactivity of released HIPVs with atmospheric oxidants. Under natural conditions, volatile exchange between plants of the same species can occur at distances up to 60 cm, while the effective response distance between individuals of different species is much smaller, at 15–20 cm (Karban et al., 2006). It has also been demonstrated that partial defoliation of *Alnus glutinosa* (L.) induced resistance to the beetle *Agelastica alni* (L.) in neighbouring plants of the same species, which declined in the plants with increased distance from defoliated trees (Dolch and Tscharntke, 2000).

Even mechanically damaged plants can release volatile signals that carry information about upcoming threats. Mechanically damaged sagebrush, *Artemisia tridentata* Nutt. induced resistance to herbivores in neighbouring plants of the same or different species (Karban et al., 2000; Karban and Shiojiri, 2009). Conspecific receivers suffered much less damage after exposure to mechanically damaged sagebrush due to accumulation of defence-related transcripts, which occur in similar ways to that observed in herbivore-attacked plants (Kessler et al., 2006). Volatile signals from genetically related individuals have a much stronger effect, in terms of reducing herbivore damage to exposed plants, than signals from less closely related plants (Karban et al., 2013).

The above examples clearly show that chemical cues from both undamaged and damaged plants induce responses in undamaged plants. In nature, most plants have to struggle with competing neighbours before they get damaged by herbivores. Therefore, it is expected that plant responses to VOCs of undamaged competitors can have an even wider ecological significance as responses to HIPVs.

6. Herbivore predator responses to volatile interactions between damaged plants

Volatiles from damaged plants can also induce responses in neighbouring plants, making them more attractive to herbivore natural enemies (Fig. 1C and D) (Dicke and Van Loon, 2000; Ninkovic et al., 2001; Kessler and Baldwin, 2002; Haddad et al., 2009; Dicke and Baldwin, 2010; Van Wijk et al., 2011). Volatiles released from infested plants are also known to induce changes in neighbouring plants, protecting them indirectly by attracting natural enemies (Bruin et al., 1992; Ninkovic et al., 2001). In a wind-tunnel experiment, unattacked Lima bean plants, *Phaseolus lunatus* L. were exposed to volatiles emitted by lima bean plants that were infested by spider-mites *Tetranychus urticae* Koch. After four to five days, the odour of Lima plants exposed to upwind of infested plants were more attractive to predatory mites *Phytoseiulus persimilis* Athias-Henriot than unexposed Lima beans. The predatory mites responded similarly to cotton plants *Gossypium hirsutum* L. treated in the same way (Dicke et al., 1990).

7. Conclusions and future prospects

Research on plant responses to volatile signals has demonstrated the capacity of plants to modify their strategies to meet a diversity of ecological challenges. Experimental evidence has shown that volatile communication between plants plays an important role in responding processes where induced plant traits contribute to mechanisms with tritrophic importance. As these changes are properties of individuals, it is necessary to scale from the level of the individual to the level of communities and ecosystems in order to understand the indirect effects of a plant's adaptive capacity. The present review has shown that volatile signals from undamaged plants mediate similar effects on tritrophic interactions as signals from herbivore-attacked plants. Nevertheless, volatile signals from intact plants have attracted less scientific attention than signals from damaged plants, which have been studied extensively during the last four decades.

With regard to the operating mechanisms, there is still a considerable lack of knowledge and understanding of the consistency of inducible systems to the developmental stage of neighbouring plants. There is currently insufficient knowledge about the limits of inducible responses in relation to necessary costs for successfully growth and reproduction. The role of inducible plant responses and allelobiotic mechanisms calls for an increased understanding of several ecological, biological and genetic aspects. Phenotypes adapted to a certain plant community may express rather epigenetic responses to surroundings that would probably occur in subsequent generations. Recent studies have shown the ability of plants to differentiate volatile signals informing them about possible threats. It is still unknown whether plants respond only to on-going threats or whether they preserve energy to react to signals predicting even more severe forthcoming threats. The more signals point to risk, the greater the chance of a real threat.

Author contributions

VN, DM and ID wrote the paper and DM constructed and drew figures.

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Plants perceive the growth pattern of their neighbours through volatiles and adapt their own growth strategy accordingly.

Iris Dahlin[1], Dimitrije Markovic[1], Robert Glinwood[1], Diana Rubene[1], Velemir Ninkovic[2]

[1]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, PO Box 7043, SE-75007 Uppsala, Sweden.

[2]Department of Ecology, Swedish University of Agricultural Sciences, PO Box 7044, SE-75007 Uppsala, Sweden.

Abstract

Very little is known about communication between undamaged plants and how this affects plant growth. We exposed one barley cultivar to volatiles of different cultivars with contrasting growth strategies (fast, intermediate, and slow growing) separately and evaluated how this affected traits of the exposed plants. We found that the growth strategy of a barley plant changed in accordance with the growth pattern of the cultivar whose volatiles it was exposed to. For most measured plant traits cultivar Luhkas had significantly higher values than the other cultivars. For exposed plants, those exposed to Luhkas also had the highest values for plant-, leaf- and stem- biomass, stem height and relative growth rate. Positive correlations between trait values of inducer and responder plants were found for the biomass of stems and roots, the leaf- and stem mass fraction, root volume, and for the length of roots with a volume higher than 1.5cm^3. The results suggest that plants are able to perceive information about the growth pattern of neighbouring plants via their volatiles, adding a new dimension to plant behaviour and community processes.

Keywords: future competition, growth pattern, plant adaptation, plant behaviour, plant communication, plant perception, plant-plant interaction, plant plasticity, volatile organic compound

Introduction

As sessile organisms, plants must adapt to a constantly changing environment. Accordingly, they have evolved mechanisms for sensing their surroundings and responding appropriately (Ballarè & Casal, 2000; Clark *et al.*, 2001; Trewavas, 2005). The identity of immediate plant neighbours may have in this sense the greatest impact for a plant, sharing available resources. When neighbour cues indicates a competitor, perceiving plants can respond with changes in their growth pattern, adjusting their physiology, morphology and phenotype accordingly (Pierik & De Wit, 2014; Kessler, 2015; Ninkovic *et al.*, 2016; Novoplansky, 2016). Plants can perceive a range of cues from neighbouring plants, such as the quality of light (Franklin, 2008), root exudates (Biedrzycki *et al.*, 2010) or volatile organic compounds (Ninkovic *et al.*, 2013). Volatile cues are important as they can reach neighbours before being shading or contact occurs (Ninkovic *et al.*, 2016).

Plants have developed a communication system to transmit information based on volatile organic compounds (Holopainen & Blande, 2012; Ueda *et al.*, 2012). Plants constantly release these volatiles and neighbouring plants can use them as a cue for sensing competitors, thus initiating growth responses that increase the competitive power of eavesdropping plants (Dicke *et al.*, 2003; Heil & Karban, 2010). During the last three decades, a substantial number of studies have investigated plant-plant communication via herbivore-induced plant volatiles (reviewed by Hare, 2011; Turlings & Erb, 2018). Far less is known about plant communication between undamaged plants and how this affect plant growth.

The ability of an individual plant to recognize neighbours, as competitors or harmless individuals is an important trait that allowing plants to adapt their competitive adjustments to a specific neighbour (Fridley *et al.,* 2007; Murphy & Dudley, 2009). Plants translate neighbour cues into the most appropriate adaptive responses by changing their allocation patterns, for example investing in height to avoid shading (Fiorucci & Fankhauser, 2017) or root growth for acquisitioning more nutrients (Craine & Dybzinski, 2013). In a recent study, barley plants grown in low red:far-red conditions developed typical shade avoidance responses (increased biomass allocation to shoots), and changed their pattern of volatile emission (Kegge *et al.*, 2015). Other barley plants grown in normal light and exposed only to volatiles from the low red:far-red plants also responded with a shade avoidance growth pattern. This indicates that plant volatile cues can have informative value about the physiological status of the emitters. Further, such cues could potentially be used by receivers to predict forthcoming stress, with responses initiated even before the stress occurs. There is currently a lack of knowledge about whether volatile substances can transmit information about the architecture and growth pattern of emitting plants, and if receiving plants can respond to such information. This is what we aimed to answer in this study.

We exposed one barley cultivar to volatiles of two different cultivars plus the same cultivar, separately, and evaluated how this affected different plant traits of the exposed plants. We hypothesize that the growth pattern of a barley cultivar changes in accordance with the growth pattern of the cultivar to whose volatiles it is exposed.

Materials and methods

Volatile exposure of one cultivar to another

Spring barley cultivars Luhkas, Salome and Fairytale (Scandinavian Seed AB, Sweden) were selected, because of their different growth strategies (fast, intermediate, and slow growing respectively) in previous field experiments (Dahlin *et al.*, paper III). The experiment consisted of three treatments – Fairytale exposed to volatiles from Fairytale, Salome and Luhkas separately – randomly placed and spatially arranged in 12 blocks. Barley seeds were germinated in Petri dishes between two filter papers for 48 hours in a climate room maintained at 18–22°C, a light regime of L16:D8 and 70% relative humidity. Light was provided by HQIE lamps (Hortilux Schréder, HPS 400 Watt, Holland) – one lamp per square meter. Germinated seeds of the same size were transferred to perforated cylindrical polyethylene tubes (1 m and 5 mm Ø) filled with washed sand (Silversand 55, Sibelco Europe, Mölndal, Sweden) and placed under the bench. Transparent two-chamber cages (each 10 x 10 x 80 cm) consisting of an inducing and receiving chamber (Ninkovic, 2003) were placed above the plant pairs. Air was sucked through an opening in the inducing chamber and volatiles from the inducing cultivar followed the airflow to the cultivar in the receiving chamber before being vented outside the room. Irrigation with tap water started two days before sowing, to establish appropriate sand moisture for plant growth. Nutrition (102 mg N, 20 mg P, 86 mg K, 8 mg S, 6 mg Ca, 8 mg MG, plus micro nutrients l-1; Wallco Cederroth International, Falun, Sweden) was provided by an automated irrigation system five days after sowing, when plants started to develop their second leaves. The light intensity at each position was measured with Apogee Quantum flux device (Apogee Instruments Inc, Logan, USA) by placing the sensor close to the plant positions. Due to limited space, the experiments were repeated three times until 24 replicates per treatment were obtained.

Sampling

Two periodic harvests were done, at 15 and 25 days after establishment of the experiments. Shoots were cut at ground level and leaves were separated from the stems. Roots were washed carefully with water, scanned in a Perspex dish filled with water and the root length and volume were calculated by using WinRHIZO Pro V 2007 software (Regent Instruments, Québec, Canada). To determine the specific leaf area (SLA), leaves were also scanned for each plant individual separately. Leaves, stems and roots were dried for 48 h at 70°C and weighed separately for each plant. To analyse biomass distribution of individual plant organs, the leaf mass fraction LMF (leaf dry mass per total plant dry mass), the stem mass fraction SMF (stem mass per total plant dry mass), and the root mass fraction RMF (root dry mass per total plant dry mass) were calculated. To quantify the speed of plant growth, the relative growth rate (RGR) was calculated as the dry mass increase per aboveground biomass per day between the two harvest time points. RGR was calculated by pairing plants: the plant with the highest biomass at 15 days after sowing (das) with the plant with the highest biomass at 25 das; and the second largest at 15 das with the second largest at 25 das, and so on (Hunt, 1987). Plant traits were measured at early growth stages, when phenotypic plasticity has most impact on plants, because phenotypic plasticity is costly and later on, plants need resources for seed production.

Statistical analyses

Differences in plant trait responses were analysed only at 25 das, because in a similar study (Ninkovic, 2003), no significant growth responses were found at 15 das.

Plant trait differences

Differences in plant traits between the inducing plants (cultivars Fairytale, Salome and Luhkas), and between the Fairytale plants exposed to VOCs from Fairytale, Salome and Luhkas were analysed with generalized linear models (GLM) with Gamma errors with a "log" link in lme4 (Bates *et al.*, 2015) in R (R Core Team, 2016). We analysed whole plant traits (biomass, and height), leaf traits (leaf biomass, leaf area and SLA), stem traits (biomass and height), root traits (biomass, length and volume) as well as LMF, SMF and RMF, the shoot to root ratio (S/R) and the RGR. One model was run for each plant trait, with cultivar (inducing cultivar for exposed plants), experiment and light intensity as fixed effects. Differences between the inducing cultivars and the responding Fairytale were analysed separately. Block was not used in the analyses due to uneven replication between experiments and because there was no spatial separation between the blocks. The factor that varied across the room – light intensity – was accounted for in the analyses.

Pairwise correlations of plant traits

The effect of the individual inducing plants (Fairytale, Salome and Luhkas) on trait values of the exposed plants (Fairytale) was analysed in pairwise correlations, i.e. each exposed plant was paired with its inducer in the data. We used GLM with Gamma errors with a "log" link, with trait value of inducing plant, experiment and light intensity as fixed effects. We did not include cultivar of the inducing plants in the analyses because of multicollinearity, as cultivars differ in their trait values. Therefor we analysed differences between cultivars and effects of inducer plants (regardless of the cultivar) separately.

Results

Descriptive growth pattern

In general, the three inducing cultivars differed in their growth pattern: Fairytale is a slow growing cultivar, with a small leave area, Salome is characterized by high SLA, and Luhkas is a fast growing cultivar, with large and heavy plants.

For most plant traits, we observed a pattern with the highest trait values for Luhkas, followed by Salome and Fairytale 25 das. Exceptions to this pattern were observed for Salome, which had the highest SLA and leaf area values, and for Fairytale, with the highest leaf mass- and root mass fraction (Table 1).

Among exposed Fairytale plants, those exposed to Luhkas had overall higher trait values 25 das except for SLA, which was highest in Fairytale exposed Fairytale (Table 2).

Plant trait differences

The inducing cultivars had different growth patterns (Fig. 1). Luhkas had significantly highest values for all plant traits compared to the other cultivars, except for root mass- and volume, and the length of roots thicker than 1.5mm (Table 1). Salome had significantly higher leaf mass- and area, and shoot to root ratio than Fairytale, and Fairytale showed a significantly higher RMF than the other cultivars at 25 das (Table 1).

Among exposed Fairytale plants, those exposed to Luhkas had significantly higher plant- leaf- and stem mass, stem height and RGR than Fairytale exposed to the other cultivars (Fig. 2; Table 2). Fairytale plants exposed to volatiles of Salome had significantly longer stems compared to Fairytale exposed Fairytale (Fig. 2). Fairytale plants exposed to volatiles of Fairytale had significant higher SLA than Fairytale exposed to the other cultivars (Table 2).

Pairwise correlations of plant traits

Positive correlations between trait values of inducer and responder plants were found for stem- and root biomass, LMF, SMF, root volume, and for the length of thick roots (volume higher than 1.5cm^3) (Fig. 3; Table 3). This means, for example, that an inducing plant with a high stem mass was associated with a higher stem mass in the responder plant. No significant correlations between inducer and responder plants were found for total plant mass, plant- and stem height, root length, leaf mass, leaf area, SLA, RMF, shoot to root ratio, and the length of roots with a volume less than 0.25cm^3 (Table 3).

Discussion

We show that plants of a particular barley cultivar express a different phenotype, when exposed to volatiles from another cultivar. The novelty of our study is that a plant responded with a similar growth pattern to the cultivar whose volatiles it was exposed to. This suggests that plants are able to perceive information about the growth pattern of neighbouring plants via volatiles, providing a novel perspective on plant behaviour and community processes.

In our study, all plants were grown under similar conditions, except for their volatile environment. Fairytale plants that shared the volatile environment with another cultivar resembled each other. To what extend such adaptive plasticity gives a competitive advantage to inducing or responding plants remains to be investigated in further experiments. For example, will coexisting plants with similar traits compete more for resources than plants with traits that differ more?

There are many examples in the literature of plastic growth responses of plants in response to neighbours when grown together, mainly through stem elongation, root growth and photosynthetic chemistry (e.g. Broz *et al.*, 2010; Chen *et al.*, 2012; Elhakeem *et al.*, 2018). In these studies, plants interact through light cues or root exudates. However, published examples of phenotypic growth responses induced by plant neighbour volatiles are rare (but see Ninkovic, 2003; Kegge *et al.*, 2015). Ninkovic (2003) found that the barley cultivar Kara exposed to volatiles from Alva allocated more biomass to roots compared with Kara exposed Kara. According to Tilman (1988), a biomass allocation to plant organs that are close to limiting resources would favour the growth of these organs. Unfortunately, the inducer plants were not measured in Ninkovic (2003), so it is unknown whether the biomass allocation observed in that study was an adaptive response to the inducer plants.

Trait values of the responding plants were in general smaller compared to traits of the inducers. However, it should be emphasized that plants were not at their final size at sampling, so our results reflect a temporal allocation of resources. Dahlin *et al.* (paper III), found that barley plants grown in cultivar mixtures developed slower early in the season and grew faster later on compared with plants grown in single cultivar stands. It would be interesting to test if the exposed plants in the current study also grow faster at later growth stages. The seeds in both studies originated from plants grown in pure stands. If this environmental information were to be expressed epigenetically, the new environment (another neighbour) might be a stress factor resulting in slow development. When this stress is permanent, the magnitude of the stress response decreases; plants habituate and can invest again in faster growth.

Positive correlations of plant traits were found for stem-, but also root related traits. If an inducer plant of any tested cultivar had a higher root volume (higher root mass and thicker roots), the exposed Fairytale plant responded with increased root growth. Larger plants need thicker roots to anchor the plant to the soil and move water and nutrients to the leaves.

Ongoing analyses and experiments are needed to reveal how plant traits of inducers and receivers developed between the two harvest time points and whether there are differences in the volatile profiles of inducing and receiving cultivars. Our model could not distinguish whether the variation in the dataset depended on the cultivar or the size of the inducing plant, because they were correlated. It will be interesting to conduct experiments in which plants are exposed to volatiles from a different cultivar but with the same size. Further, it would be interesting to test whether exposure to volatiles from an inducer cultivar that is shorter than the responding plants also results in growth responses. In this case, our hypothesis is that plants of a certain height respond to volatiles from shorter plants with similar adaptive growth responses as when exposed to longer plants. When a plant neighbour is shorter than the focal plant, there is no competition for light and therefore no reason to invest in growth. Regardless of whether it is the cultivar or the size of the inducer that resulted in changed growth responses in exposed plants, the results suggest that responding plants have the ability to discriminate between volatile signals from different neighbours.

The current results add a new dimension to plant coexistence, showing that volatiles of different plants can induce growth changes in neighbouring plants. Such interactions should be taken into account in studies that investigate the traits of plants growing in the neighbourhood of other plants.

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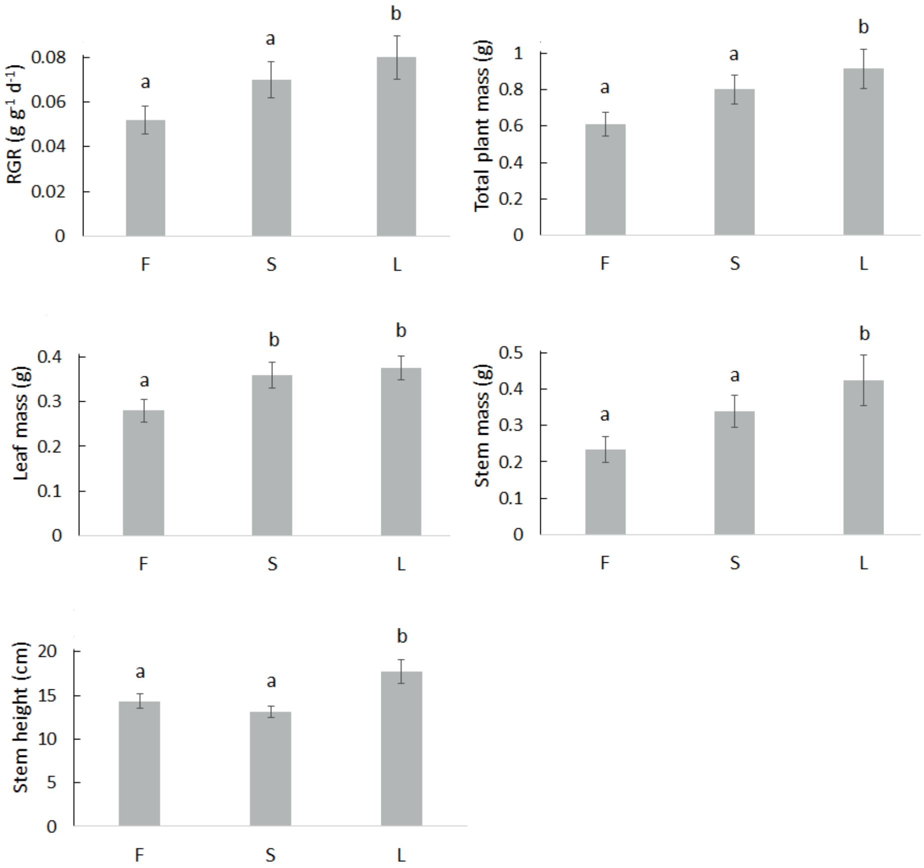

Figure 1. Mean plant trait values of the inducing cultivars Fairytale (F), Salome (S) and Luhkas (L), 25 days after sowing. Significant differences ($P < 0.05$) are indicated by different letters.

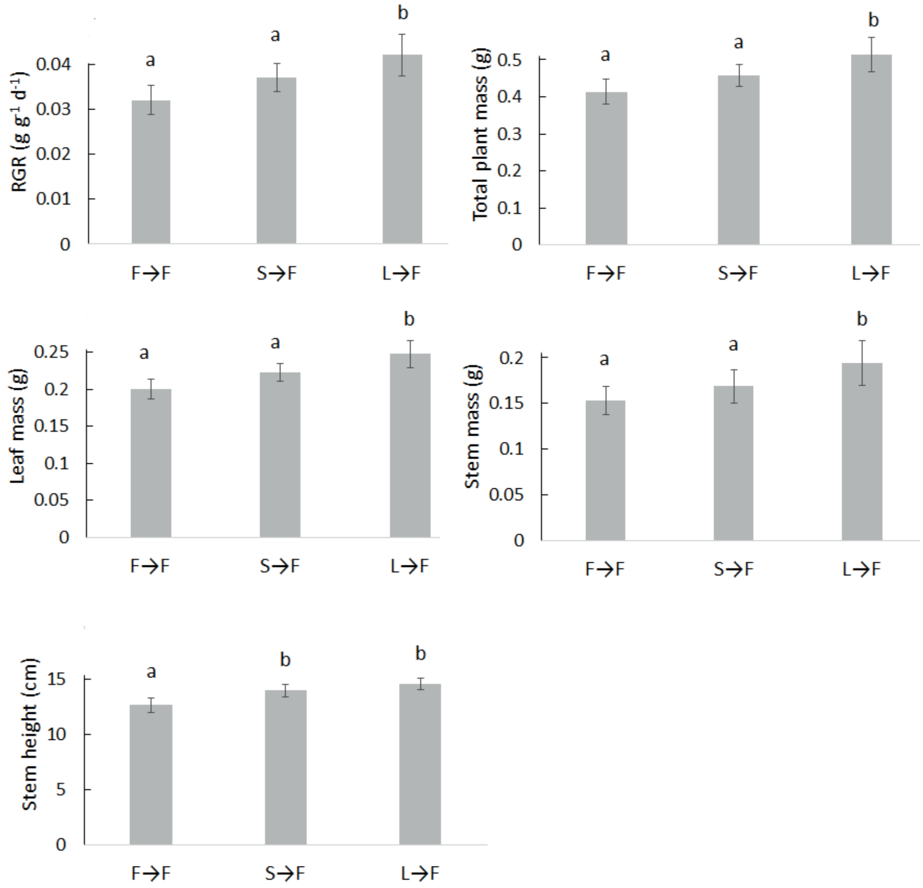

Figure 2. Mean plant trait values of cultivar Fairytale exposed to volatiles of Fairytale (F→F), Salome (S→F), and Luhkas (L→F), 25 days after sowing. Significant differences (P < 0.05) are indicated by different letters.

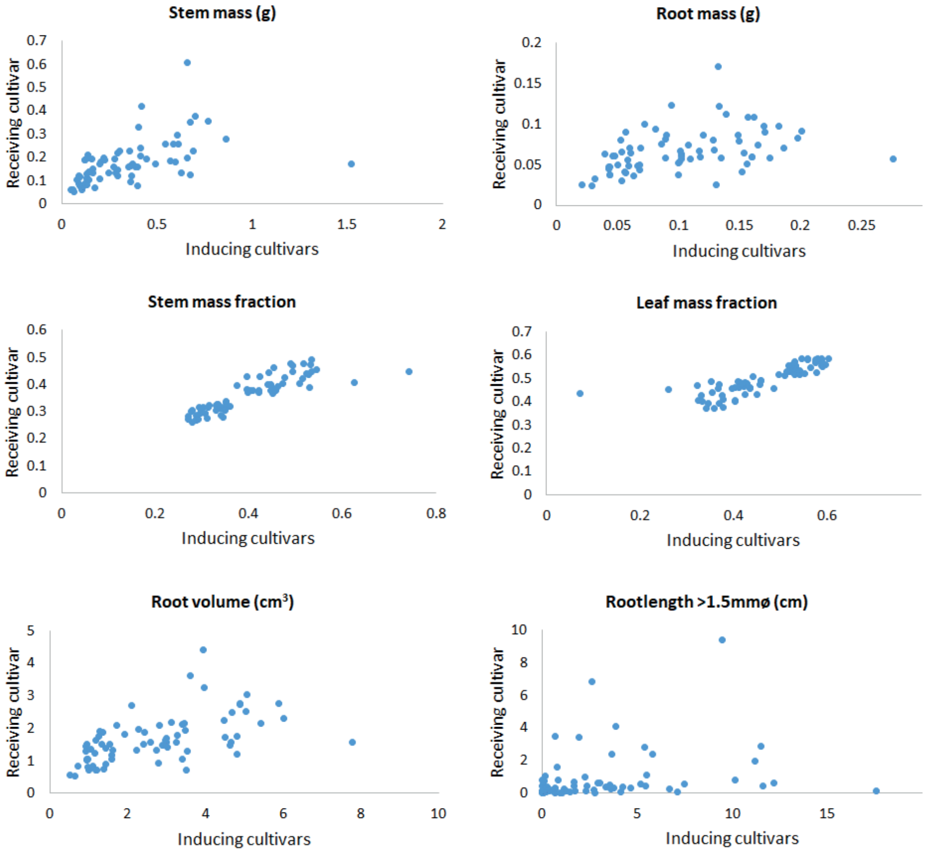

Figure 3. Pairwise correlations of the effect of individual inducing plants (Fairytale, Salome and Luhkas) on trait values of receiver plants (Fairytale), 25 days after sowing.

Table 1. Generalised linear model output (Estimates, Standard error, t- and p-value) testing differences of plant traits of inducing cultivars Fairytale, Salome and Luhkas, 25 days after sowing, n = 24. Cultivar Fairytale was used as reference, and cultivar, light intensity and experiment as fixed effects. Significance codes (* p<0.05; ** p<0.01; ***p<0.001).

	Intercept	Salome	Lukas	Light intensity	Experiment 2	Experiment 3
Total plant mass (g)						
Est. ± SE	-1.24 ± 0.15	0.17 ± 0.11	0.29 ± 0.11	0.09 ± 0.07	0.46 ± 0.13	0.98 ± 0.10
t-value	-8.23	1.52	3.59	1.27	3.66	9.00
Pr (>|t|)	1.2 e^{-11}***	0.13	0.01*	0.21	0.0005***	4 e^{-13}***
Stem mass (g)						
Est. ± SE	-2.62 ± 0.16	0.23 ± 0.12	0.43 ± 0.12	0.11 ± 0.08	0.66 ± 0.14	1.55 ± 0.12
t-value	-16.36	1.97	3.64	1.40	4.90	13.3
Pr (>|t|)	< 2 e^{-16}***	0.05	0.0005***	0.17	7 e^{-6}***	< 2 e^{-16}***
Leaf mass (g)						
Est. ± SE	-1.89 ± 0.13	0.24 ± 0.10	0.28 ± 0.10	0.11 ± 0.06	0.44 ± 0.11	0.65 ± 0.09
t-value	-14.55	2.46	2.94	1.73	3.97	6.85
Pr (>|t|)	< 2 e^{-16}***	0.02*	0.005**	0.09	0.0002***	3 e^{-9}***
Root mass (g)						
Est. ± SE	-3.24 ± 0.14	0.07 ± 0.10	0.17 ± 0.11	0.10 ± 0.07	0.55 ± 0.12	1.02 ± 0.10
t-value	-22.84	0.65	1.65	1.50	4.62	9.88
Pr (>|t|)	< 2 e^{-16}***	0.52	0.10	0.14	2 e^{-5}***	1 e^{-14}***
Plant height (cm)						
Est. ± SE	3.81 ± 0.04	-0.005 ±0.03	0.07 ± 0.03	0.02 ± 0.02	0.09 ± 0.03	0.31 ± 0.03
t-value	110.0	-0.21	2.75	1.40	2.92	12.4
Pr (>|t|)	< 2 e^{-16}***	0.83	0.008**	0.17	0.005**	2 e^{-16}***
Stem height (cm)						
Est. ± SE	2.46 ± 0.06	-0.09 ± 0.05	0.11 ± 0.05	0.07 ± 0.03	0.24 ± 0.04	-
t-value	41.35	-1.93	2.16	1.76	6.04	-
Pr (>|t|)	< 2 e^{-16}***	0.06	0.04*	0.08	1 e^{-6}***	-
Leaf area (cm^2)						
Est. ± SE	4.16 ± 0.11	0.27 ±0.08	0.17 ± 0.08	0.09 ± 0.05	0.27 ± 0.09	0.26 ± 0.08
t-value	39.17	3.43	2.15	1.79	3.02	3.36
Pr (>|t|)	< 2 e^{-16}***	0.001**	0.04*	0.08	0.004**	0.001**
Root length (cm)						
Est. ± SE	6.95 ± 0.13	0.09 ± 0.10	0.34 ± 0.10	0.11 ± 0.06	0.64 ± 0.11	1.10 ± 0.10
t-value	53.17	0.92	3.45	1.75	5.80	11.5
Pr (>|t|)	< 2 e^{-16}***	0.36	0.001***	0.09	2 e^{-7}***	< 2 e^{-16}***
Root length < 0.25mm ø (cm)						
Est. ± SE	6.32 ± 0.13	0.12 ± 0.10	0.43 ± 0.10	0.11 ± 0.07	0.72 ± 0.11	0.95 ± 0.10
t-value	47.41	1.18	4.35	1.69	6.42	9.76
Pr (>|t|)	< 2 e^{-16}***	0.24	5 e^{-5}***	0.10	2 e^{-8}***	2 e^{-14}***
Root length > 1.5mm ø (cm)						
Est. ± SE	-2.15 ± 0.41	0.27 ± 0.30	0.59 ± 0.30	0.46 ± 0.20	2.42 ± 0.34	3.54 ± 0.30
t-value	-5.30	0.91	1.97	2.30	7.05	12.0
Pr (>|t|)	1.5 e^{-6}***	0.36	0.05	0.03*	1 e^{-9}***	< 2 e^{-16}***
Root volume (cm^3)						
Est. ± SE	-0.16 ± 0.15	0.07 ± 0.11	0.21 ± 0.11	0.11 ± 0.07	0.55 ± 0.12	1.27 ± 0.10
t-value	-1.10	0.67	1.97	1.55	4.45	12.0
Pr (>|t|)	0.28	0.50	0.05	0.13	3 e^{-5}***	< 2 e^{-16}***
Specific leaf area (cm^2 g^{-1})						
Est. ± SE	6.08 ± 0.04	0.006 ± 0.03	-0.13 ± 0.03	-0.01 ± 0.02	-0.16 ± 0.04	-0.39 ± 0.03
t-value	145.5	0.18	-4.07	-0.68	-4.60	-12.8

	Intercept	Salome	Lukas	Light intensity	Experiment 2	Experiment 3
Pr (>|t|)	**< 2 e^{-16}*** **	0.86	**0.0001*** **	0.50	**2 e^{-5}*** **	**< 2 e^{-16}*** **
Stem mass fraction						
Est. ± SE	-1.34 ± 0.05	0.06 ± 0.03	0.14 ± 0.03	0.001 ± 0.02	0.18 ± 0.04	0.54 ± 0.03
t-value	-29.70	1.77	4.09	0.06	4.63	16.3
Pr (>|t|)	**< 2 e^{-16}*** **	0.08	**0.0001*** **	0.96	**2 e^{-5}*** **	**< 2 e^{-16}*** **
Leaf mass fraction						
Est. ± SE	-0.52 ± 0.06	-0.006 ±0.04	-0.11 ± 0.04	0.02 ± 0.03	-0.10 ± 0.05	-0.42 ± 0.04
t-value	-9.20	-0.14	-2.57	0.54	-2.2	-10.3
Pr (>|t|)	**2 e^{-13}*** **	0.89	**0.01* **	0.59	**0.03* **	**2 e^{-15}*** **
Root mass fraction						
Est. ± SE	-1.93 ± 0.05	-0.11 ± 0.04	-0.11 ± 0.04	-0.01 ± 0.03	0.04 ± 0.04	0.01 ± 0.04
t-value	-38.06	-3.02	-2.82	-0.54	0.88	0.32
Pr (>|t|)	**< 2 e^{-16}*** **	**0.004** **	**0.007** **	0.59	0.38	0.75
Shoot to root ratio						
Est. ± SE	1.72 ± 0.05	0.15 ± 0.03	0.16 ± 0.03	0.02 ± 0.02	-0.02 ± 0.04	0.02 ± 0.03
t-value	38.10	4.59	4.68	0.93	-0.43	0.51
Pr (>|t|)	**< 2 e^{-16}*** **	**2 e^{-5}*** **	**1.5 e^{-5}*** **	0.36	0.67	0.61
Relative growth rate (g g^{-1} d^{-1})						
Est. ± SE	-2.95 ± 0.12	0.28 ± 0.16	0.39 ± 0.17	-	-	-
t-value	-25.4	1.72	2.42	-	-	-
Pr (>|t|)	**< 2 e^{-16}*** **	0.09	**0.02* **	-	-	-

Table 2. Generalised linear model output (Estimates, Standard error, t- and p-value) testing differences of plant traits of cultivar Fairytale exposed to volatiles of Fairytale (F > F), Salome (S > F) and Luhkas (L > F), 25 days after sowing, n = 24. Cultivar F > F was used as reference, and cultivar, light intensity and experiment as fixed effects. Significance codes (* p<0.05; ** p<0.01; ***p<0.001).

	Intercept	S > F	L > F	Light intensity	Experiment 2	Experiment 3
Total plant mass (g)						
Est. ± SE	-1.42 ± 0.13	0.11 ± 0.10	0.22 ± 0.10	0.09 ± 0.07	0.44 ± 0.11	0.54 ± 0.10
t-value	-10.86	1.16	2.24	1.41	4.01	5.72
Pr (>|t|)	**2 e^{-16}*** **	0.25	**0.03* **	0.16	**0.0002*** **	**3 e^{-7}*** **
Stem mass (g)						
Est. ± SE	-2.70 ± 0.15	0.13 ± 0.11	0.25 ± 0.11	0.09 ± 0.07	0.56 ± 0.13	0.96 ± 0.11
t-value	-18.28	1.17	2.26	1.20	4.49	8.93
Pr (>|t|)	**< 2 e^{-16}*** **	0.25	**0.03* **	0.24	**3 e^{-5}*** **	**6 e^{-13}*** **
Leaf mass (g)						
Est. ± SE	-1.97 ± 0.17	0.10 ± 0.09	0.19 ± 0.09	0.09 ± 0.06	0.38 ± 0.10	0.27 ± 0.08
t-value	-16.89	1.14	2.22	1.61	3.86	3.19
Pr (>|t|)	**< 2 e^{-16}*** **	0.26	**0.03* **	0.11	**0.0003*** **	**0.002** **
Root mass (g)						
Est. ± SE	-3.32 ± 0.14	0.11 ± 0.10	0.20 ± 0.10	0.09 ± 0.07	0.44 ± 0.12	0.48 ± 0.10
t-value	-23.52	1.04	1.94	1.29	3.65	4.65
Pr (>|t|)	**< 2 e^{-16}*** **	0.30	0.06	0.20	**0.0005*** **	**2 e^{-5}*** **
Plant height (cm)						
Est. ± SE	3.80 ± 0.04	0.05 ± 0.03	0.04 ± 0.03	0.02 ± 0.02	0.12 ± 0.03	0.24 ± 0.03
t-value	107.34	1.78	1.67	0.85	3.82	9.27

	Intercept	S > F	L > F	Light intensity	Experiment 2	Experiment 3
Pr (>|t|)	< 2 e^{-16}***	0.08	0.10	0.40	0.0003***	2 e^{-13}***
Stem height (cm)						
Est. ± SE	2.39 ± 0.06	0.10 ± 0.05	0.13 ± 0.05	0.03 ± 0.03	0.20 ± 0.04	-
t-value	42.55	2.13	2.87	1.01	5.15	-
Pr (>|t|)	< 2 e^{-16}***	0.04*	0.007**	0.32	1 e^{-5}***	-
Leaf area (cm^2)						
Est. ± SE	4.04 ± 0.09	0.08 ± 0.07	0.13 ± 0.07	0.07 ± 0.04	0.27 ± 0.08	0.08 ± 0.07
t-value	45.23	1.15	1.89	1.59	3.58	1.16
Pr (>|t|)	< 2 e^{-16}***	0.25	0.06	0.12	0.0007***	0.25
Root length (cm)						
Est. ± SE	6.94 ± 0.14	0.10 ± 0.10	0.14 ± 0.10	0.13 ± 0.07	0.44 ± 0.11	0.53 ± 0.10
t-value	51.51	1.05	1.42	1.98	3.80	5.36
Pr (>|t|)	< 2 e^{-16}***	0.30	0.16	0.05	0.0003***	1 e^{-6}***
Root length < 0.25mm ø (cm)						
Est. ± SE	6.36 ± 0.14	0.11 ± 0.10	0.14 ± 0.10	0.14 ± 0.07	0.49 ± 0.12	0.37 ± 0.10
t-value	45.43	1.09	1.32	2.06	4.16	3.58
Pr (>|t|)	< 2 e^{-16}***	0.28	0.19	0.04*	10 e^{-5}***	0.0007***
Root length > 1.5mm ø (cm)						
Est. ± SE	-1.47 ± 0.61	0.11 ± 0.45	0.68 ± 0.45	0.20 ± 0.30	1.66 ± 0.52	1.61 ± 0.45
t-value	-2.40	0.23	1.50	0.65	3.19	3.61
Pr (>|t|)	0.02*	0.82	0.14	0.52	0.002**	0.0006***
Root volume (cm^3)						
Est. ± SE	-0.24 ± 0.14	0.09 ± 0.10	0.16 ± 0.11	0.12 ± 0.07	0.40 ± 0.12	0.70 ± 0.10
t-value	-1.65	0.82	1.49	1.65	3.33	6.70
Pr (>|t|)	0.10	0.41	0.14	0.10	0.001**	6 e^{-9}***
Specific leaf area (cm^2 g^{-1})						
Est. ± SE	6.02 ± 0.04	-0.03 ± 0.03	-0.06 ± 0.03	-0.02 ± 0.02	-0.11 ± 0.03	-0.18 ± 0.03
t-value	149.9	-0.92	-1.86	-1.11	-3.27	-6.32
Pr (>|t|)	< 2 e^{-16}***	0.36	0.07	0.27	0.002**	3 e^{-8}***
Stem mass fraction						
Est. ± SE	-1.27 ± 0.03	0.01 ± 0.02	0.03 ± 0.02	-0.003 ± 0.01	0.11 ± 0.03	0.39 ± 0.02
t-value	-43.73	0.45	1.34	-0.24	4.58	18.39
Pr (>|t|)	< 2 e^{-16}***	0.66	0.19	0.81	2 e^{-5}***	< 2 e^{-16}***
Leaf mass fraction						
Est. ± SE	-0.56 ± 0.03	-0.007 ±0.02	-0.02 ± 0.02	0.003 ± 0.01	-0.06 ± 0.02	-0.25 ± 0.02
t-value	-21.53	-0.38	-0.84	0.24	-2.83	-13.3
Pr (>|t|)	< 2 e^{-16}***	0.71	0.41	0.81	0.006**	< 2 e^{-16}***
Root mass fraction						
Est. ± SE	-1.90 ± 0.04	0.001 ± 0.03	-0.02 ± 0.03	-0.007 ± 0.02	0.004 ± 0.03	-0.06 ± 0.03
t-value	-48.47	0.05	-0.76	-0.34	0.12	-1.94
Pr (>|t|)	< 2 e^{-16}***	0.96	0.45	0.74	0.91	0.06
Shoot to root ratio						
Est. ± SE	1.75 ± 0.05	-0.0008±0.03	0.03 ± 0.03	0.01 ± 0.02	-0.002 ± 0.04	0.06 ± 0.03
t-value	38.99	-0.03	0.79	0.47	-0.06	1.94
Pr (>|t|)	< 2 e^{-16}***	0.98	0.43	0.64	0.95	0.06
Relative growth rate (g g^{-1} d^{-1})						
Est. ± SE	-3.46 ± 0.08	0.16 ± 0.11	0.29 ± 0.11	-	-	-
t-value	-43.4	1.41	2.57	-	-	-
Pr (>|t|)	< 2 e^{-16}***	0.16	0.01*	-	-	-

Table 3. Generalised linear model output (Estimates, Standard error, t-and p-value) testing the effect of inducer plant trait values on trait values of the receiving plants in pairwise comparisons, 25 days after sowing, n = 24. Significance of difference (* p<0.05; ** p<0.01; *** p<0.001) is marked in bold.

	Intercept	Inducer trait value	Light intensity	Experiment 2	Experiment 3
Total plant mass (g)					
Est. ± SE	-1.40 ± 0.14	0.24 ± 0.12	0.10 ± 0.07	0.38 ± 0.12	0.37 ± 0.13
t-value	-10.35	1.96	1.52	3.19	2.89
Pr (>|t|)	**2 e^{-15}*****	0.05	0.13	**0.002****	**0.005****
Stem mass (g)					
Est. ± SE	-2.64 ± 0.15	0.55 ± 0.24	0.10 ± 0.07	0.50 ± 0.13	0.72 ± 0.15
t-value	-17.85	2.26	1.42	3.80	4.92
Pr (>|t|)	**< 2 e^{-16}*****	**0.03***	0.16	**0.0003*****	**6 e^{-6}*****
Leaf mass (g)					
Est. ± SE	-1.96 ± 0.13	0.46 ± 0.29	0.10 ± 0.06	0.33 ± 0.11	0.18 ± 0.10
t-value	-15.71	1.58	1.63	3.03	1.77
Pr (>|t|)	**< 2 e^{-16}*****	0.12	0.11	**0.004****	0.08
Root mass (g)					
Est. ± SE	-3.34 ± 0.15	2.66 ± 1.15	0.09 ± 0.07	0.33 ± 0.13	0.23 ± 0.15
t-value	-22.57	2.32	1.31	2.58	1.57
Pr (>|t|)	**< 2 e^{-16}*****	**0.02***	0.19	**0.01***	0.12
Plant height (cm)					
Est. ± SE	3.91 ± 0.09	-0.002 ± 0.002	0.02 ± 0.02	0.12 ± 0.03	0.27 ± 0.04
t-value	45.98	-1.10	1.35	3.74	6.59
Pr (>|t|)	**< 2 e^{-16}*****	0.28	0.18	**0.0004*****	**9 e^{-9}*****
Stem height (cm)					
Est. ± SE	2.53 ± 0.12	-0.006 ± 0.01	0.05 ± 0.03	0.21 ± 0.06	-
t-value	20.73	-0.62	1.48	3.78	-
Pr (>|t|)	**< 2 e^{-16}*****	0.54	0.15	**0.0007*****	-
Leaf area (cm^2)					
Est. ± SE	4.04 ± 0.11	0.0009 ±0.0009	0.08 ±0.05	0.25 ± 0.08	0.05 ± 0.07
t-value	36.63	0.93	1.65	2.98	0.66
Pr (>|t|)	**< 2 e^{-16}*****	0.36	0.10	**0.004****	0.51
Root length (cm)					
Est. ± SE	7 e^{+0} ± 1 e^{-1}	6 e^{-5} ± 3 e^{-5}	1 e^{-1} ± 7 e^{-2}	3 e^{-1} ± 1 e^{-1}	3 e^{-1} ± 1 e^{-1}
t-value	50.80	1.85	1.92	2.82	2.41
Pr (>|t|)	**< 2 e^{-16}*****	0.07	0.06	**0.007****	**0.02***
Root length <0.25mm ø (cm)					
Est. ± SE	6 e^{+0} ± 1 e^{-1}	8 e^{-5} ± 7 e^{-5}	1 e^{-1} ±7 e^{-2}	4 e^{-1} ± 1 e^{-1}	2 e^{-1} ± 1 e^{-1}
t-value	44.69	1.33	2.06	3.14	1.82
Pr (>|t|)	**< 2 e^{-16}*****	0.19	**0.04***	**0.003****	0.07
Root length > 1.5mm ø (cm)					
Est. ± SE	-2.00 ± 0.58	0.18 ± 0.06	0.27 ± 0.29	1.42 ± 0.51	0.60 ± 0.51
t-value	-3.47	3.13	0.94	2.77	1.17
Pr (>|t|)	**0.0009*****	**0.003****	0.35	**0.007****	0.25
Root volume (cm^3)					
Est. ± SE	-0.23 ± 0.14	0.10 ± 0.04	0.11 ± 0.07	0.32 ± 0.13	0.41 ± 0.15
t-value	-1.63	2.36	1.60	2.51	2.63
Pr (>|t|)	0.11	**0.02***	0.12	**0.02***	**0.01***
Specific leaf area (cm^2 g^{-1})					
Est. ± SE	6 e^{+0} ± 5 e^{-2}	4 e^{-5} ± 4 e^{-5}	-3 e^{-2} ± 2 e^{-2}	-1 e^{-1} ± 4 e^{-2}	-2 e^{-1} ± 3 e^{-2}
t-value	133.1	0.90	-1.31	-3..05	-5.91

	Intercept	Inducer trait value	Light intensity	Experiment 2	Experiment 3
Pr (>|t|)	**< 2 e $^{-16}$*****	0.37	0.20	**0.003****	**1 e $^{-7}$*****
			Stem mass fraction		
Est. ± SE	-1.43 ± 0.05	0.62 ± 0.15	-0.004 ± 0.01	0.08 ± 0.02	0.27 ± 0.04
t-value	-28.54	4.09	-0.32	3.34	7.68
Pr (>|t|)	**< 2 e $^{-16}$*****	**0.0001*****	0.75	**0.001****	**10 e $^{-11}$*****
			Leaf mass fraction		
Est. ± SE	-0.76 ± 0.09	0.35 ± 0.14	0.0003 ± 0.01	-0.04 ± 0.02	-0.18 ± 0.03
t-value	-9.02	2.43	0.02	-1.93	-5.53
Pr (>|t|)	**4 e $^{-13}$*****	**0.02***	0.98	0.06	**6 e $^{-7}$*****
			Root mass fraction		
Est. ± SE	-1.90 ± 0.08	-0.026 ± 0.47	-0.009 ± 0.02	0.003 ± 0.02	-0.055 ± 0.03
t-value	-24.0	-0.06	-0.47	0.10	-1.87
Pr (>|t|)	**< 2 e $^{-16}$*****	0.96	0.64	0.92	0.07
			Shoot to root ratio		
Est. ± SE	1.73 ± 0.09	0.003 ± 0.01	0.01 ± 0.02	-0.002 ± 0.04	0.06 ± 0.03
t-value	18.45	0.25	0.51	-0.05	1.90
Pr (>|t|)	**< 2 e $^{-16}$*****	0.81	0.61	0.96	0.06

III

Plasticity of Barley in Response to Plant Neighbours in Cultivar Mixtures

Iris Dahlin[1], Lars P Kiær[2], Göran Bergkvist[1], Martin Weih[1], Velemir Ninkovic[3]

[1]Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

[2]Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark.

[3]Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Abstract

Little is known about how cultivars grown in mixture change phenotypically, and how this affects mixture performance. The altered local environment in cultivar mixtures can result in plastic responses of plant functional traits, potentially increasing or decreasing individual fitness. We aim to determine if spring barley cultivars show different phenotypic trait responses when grown in mixtures with different cultivars, whether these responses depend on the neighbouring cultivar identity and how this contributes to productivity. Five cultivars were grown in pure stands and in four pairwise mixtures in two years field experiments. Plant traits related to development, growth, nitrogen economy, and reproduction were measured to identify temporal patterns of genotypic variation and plastic response to neighbouring plants. Evidence was found for plastic changes in growth related traits of cultivars grown in mixtures compared to pure stands. Plants in cultivar mixtures developed slower early in the season, and grew faster later on. In some mixtures, higher nitrogen uptake and accumulation efficiency were found, as well as higher grain and biomass production. Competitive release through niche differentiation and resource partitioning can explain the increased grain and biomass production in some cultivar combinations. This study contributes to understanding how productivity in cultivar mixtures may be affected by plastic adaptation of plant functional traits, depending on the environment created by neighbouring genotypes. We show that morphological, physiological and phenological characteristics of barley cultivars tend to change when grown with other cultivars, becoming more similar than traits of plants grown in pure stands. We show that the lowest possible level of botanical diversity in agricultural fields can significantly affect plant growth and development, depending on the cultivar neighbourhood. The results indicate that plastic interactions between cultivars might be an important driver behind the variability in reported mixing effects of previous studies. Investigations of individual plant trait responses to various cultivar environments are needed to identify the most promising cultivar mixtures and to tailor more efficient, stable and predictable cropping systems.

Keywords: adaptive plasticity, biodiversity, biomass allocation, competition, cultivar mixture, genotype, phenotype, productivity

Introduction

Genotypic diversity in agriculture can promote vegetative and reproductive yield (Cook-Patton *et al.*, 2011; Reiss & Drinkwater, 2017). However, reviews and meta-analyses have revealed substantial variability of these functions in crops – genotypic diversity did not always give higher yields than their component pure stands (Kiær *et al.*, 2009; Reiss & Drinkwater, 2017; Borg *et al.*, 2018). Therefore, a better understanding of the processes underlying crop genotypic diversity on productivity is needed in order to (i) explain why some studies find positive effects whereas others do not, and (ii) enable predictive development of sustainable agricultural cropping systems based on genotype diversity.

Productive agriculture usually depends on large amounts of nitrogenous fertilizers, which are expensive and energy consuming, and their use is often associated with great environmental risks (e.g., leaching to groundwater, climate gas emissions) and decreased ecological sustainability in agriculture. An improvement of nitrogen (N) uptake and use efficiency of crop plants is thus of key importance for maintaining productivity and at the same time improve sustainability in agriculture. Though stated in many studies that productivity is improved in cultivar mixtures through complementary, compensation or facilitation, it has not been examined in detail how N uptake and use efficiency is affected (e.g. Brooker *et al.*, 2008; Creissen *et al.*, 2013).

Functional traits are morphological, physiological and phenological aspects modulating plant performance via their effects on growth, survival and reproductive output (Violle *et al.,* 2007). Most plant traits are generally considered to be plastic (Schlichting, 2002), thereby allowing single genotypes to adapt to local environmental conditions by producing differential phenotypes. Trait plasticity has been shown to increase (Cahill & McNickle, 2011, Ninkovic *et al.*, 2016; Novoplansky, 2016) or decrease (Grenier *et al.*, 2016) individual fitness. In this study, we define plasticity as a genotype-dependent characteristic, varying between crop cultivars and different environmental stimuli. In this case, the growth environment was changed through the choice of the neighbouring cultivars. A large amount of studies has investigated phenotypic plasticity in response to environmental factors and in response to plant neighbours of other species (reviewed by Gratani, 2014). In spite of the altered environments present in cultivar mixtures compared to pure stands (e.g. light regimes in the altered canopies), very little is known about trait plasticity in cultivar mixtures and the extent to which this affects plant growth and development, and in turn cultivar interaction and mixture performance. Essah and Stoskopf (2001) studied mixture performance of barley cultivars with different plant heights. They found a yield advantage compared with pure stands, but not in all combinations. Further, they did not measure if plants responded to mixing with plastic responses, which could have explained why not all mixtures were more productive in their study. In this study, we assessed a wide range of plant traits of cultivars grown in mixtures and pure stand, covering plant growth, vigor, stretching, development, nitrogen economy and reproduction. Yet, understanding plastic responses that allow cultivars to reallocate biomass is fundamentally important to explain diversity-productivity relationships and significant for designing sustainable and productive cropping systems.

To study plastic responses of functional traits in barley, we manipulated barley diversity in two successive field experiments, in which cultivars were grown in plots of pure stands and in two-cultivar mixtures in alternate-rows, enabling cultivar identification of individual plants for measurements and sampling. This made it possible to evaluate individual plant traits at cultivar level rather than stand level, and their interactions with neighbouring cultivars. With the idea in mind that phenotypic responses to neighbouring plants are dynamic, several plant traits were measured at different occasions to identify developmental plasticity of barley plants in response to plant neighbourhood.

The aim of this study was to determine if a specific cultivar shows different phenotypic responses in functional traits when grown with another cultivar in alternate rows and if this varies dependent on the component cultivars. We hypothesize that

- biomass and grain production is promoted by mixtures (*overall effect of mixing*),
- traits of individual cultivars differ when grown in a mixture as compared to a pure stand (*plastic trait response to mixing*),
- plastic trait responses depend on the cultivar neighbourhood of the focal plant individual (*neighbourhood effect*, i.e. the site of plant neighbours growing beside it), and
- traits and properties of two cultivars become more similar when the plants grow in a mixture (*adaptive similarity*, i.e. release from competition-derived niche reinforcement).

Materials and methods

Field experiments

Two field trials were conducted in Central-Eastern Sweden at the Lövsta field research station (59°52′N, 17°48′E) of the Swedish University of Agricultural Sciences in 2015 and 2016. The five spring barley (*Hordeum vulgare* L.) cultivars Salome, Fairytale, Rosalina, Anakin and Luhkas (Table 1) were grown in pure stands and in two-cultivar combinations. The cultivar Salome was included as a component in all mixtures because it is known to induce plant responses in other cultivars (Dahlin *et al.*, 2018). The fields were sown in the beginning of May with 400 seeds m^{-2} and a row spacing of 12.5 cm. Each of the nine treatment plots (3 ×9 m) represented a replicate and the plots were placed in in a randomized complete block design with six blocks, having a distance of 1 m between plots. The corridors between the plots were unsown and regularly weeded by hand.

Acquisition of trait data

A wide range of plant traits was assessed in each plot, covering plant development, growth and vigor, stretching, nitrogen economy and reproduction. The number of tillers per plant was estimated along two representative meter transects following the rows in each plot by counting the number of plants before the onset of tillering. After BBCH 29 (Lancashire *et al.*, 1991), the number of tillers on the same transects were counted and divided by the original number of plants, providing estimates of the number of tillers per plant. As a measure of plant development, the growth stage of each of five randomly chosen plants per cultivar per plot was determined 13 July 2015 (52 days after sowing) and 28 June 2016 (47 days after sowing). In addition, the average growth stage across treatments was recorded on the day of all other trait assessments. The leaf chlorophyll content was assessed in five randomly selected plants per cultivar per plot. Three leaves (the first to the third leaf measured downwards) of each plant were measured, using a SPAD-502 chlorophyll meter (Konica Minolta sensing inc, Japan). This was done at flowering (BBCH 61) in 2015 and at emergence of the flag leaf (BBCH 40) in 2016. The selected plants were then cut at soil surface, and the three leaves were detached and scanned individually, using a flatbed scanner (EPSON Perfection 4900 3.4, Regent Instruments, Quebec, Canada). Leaf area was then quantified, using Win-RHIZO Pro V 2007 software (Regent Instruments, Quebec, Canada). Those leaves were also weighed, after drying at 70°C for 24h and the specific leaf area was calculated. Biomass production was assessed by destructive sampling of ten randomly chosen plants per cultivar per plot at three time points: when first tiller was visible (BBCH 21), at early flowering (BBCH 61) and at maturity (BBCH 87). Plant material was dried at 70 °C for 24 h before measuring the dry weight. The plant height was measured manually from the soil surface to the end of the upper leaf sheath (Pérez-Harguindeguy *et al.*, 2013) on ten randomly chosen plants per cultivar per plot. This was done at advanced tillering (BBCH 24, at this time point it was the leaf length measured), booting (BBCH 47) and early flowering (BBCH 61) in 2015, as well as at jointing (BBCH 31) and emergence of the flag leaf (BBCH 40) in 2016. The number and height of nodes of these plants were also recorded. Nitrogen content and grain mass were assessed on plants along the above mentioned transects which were harvested by cutting at soil surface at maturity (BBCH 87). Following oven drying at 70 °C for 24 h, the green biomass was ground with a ball mill (MM 400, Retsch, Haan, Germany) and the N concentrations were analysed by Near Infrared Transmittance (InfratecTM 1241 Grain Analyser, FOSS, Hillerød, Denmark). Barley heads were threshed, using a stationary research thresher/blower (Siemens-Schuckert, Germany) and estimates of grain mass and N content in the grains were recorded, using the same method for N content in the grains as for leaf N content. The time points of measurements and sampling differ for the two years due to limiting weather conditions.

Calculations

To quantify the speed of plant growth, relative growth rate (RGR, d^{-1}) was calculated as the increase in aboveground biomass per day, using the following equation (Hoffmann & Poorter, 2002):

$$RGR = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)} \quad \text{(eq1)}$$

where W_1 and W_2 are mean aboveground plant dry weights at times t_1 and t_2, respectively. The RGR was also quantified for the growth of plant height, and referred to as relative stretching rate.

The specific leaf area (SLA, m^2 g^{-1}), used to estimate a component of leaf biomass allocation, was calculated as the leaf area per leaf weight (Vile *et al.*, 2005):

$$SLA = \frac{leaf\ area\ m^2}{leaf\ weight\ g} \quad \text{(eq2)}$$

The plant N economy was assessed using a plant-based approach developed by Weih *et al.* (2011), with terminology modified by Weih *et al.* (2018). Accordingly, overall plant N accumulation efficiency (NAE) is the N amount in the produced grain per unit N amount in the sown grains (g g^{-1}), broken down into three components as shown in equation 3.

$$NAE = U_N \cdot E_{N,g} \cdot C_{N,g} \quad \text{(eq3)}$$

where the mean N uptake efficiency (U_N; g g^{-1}) is obtained as the mean N amount per plant during the growth period per N amount in the seed grain. The grain-specific N efficiency ($E_{N,g}$; g g^{-1}) is obtained as the biomass of harvested grain divided by the mean N amount per plant accumulated during the growth period, which reflects the grains produced at final harvest per mean plant N content (or N productivity). The grain N concentration at final harvest ($C_{N,g}$; g g^{-1}) is obtained as the N carry-over from the whole plant to the grains (the grain N allocation divided by the grain biomass allocation at final harvest) (Weih *et al*., 2011).

Differences in the final dry matter partition (harvest index, HI) were calculated as the ratio of harvested grain biomass to total aboveground biomass (Gifford *et al.*, 1984):

$$HI = \frac{grain\ yield}{biomass\ yield} \qquad \text{(eq4)}$$

Statistical analysis

Mixed linear models were employed for most statistical analyses, using the nlme package in *R* (Pinheiro *et al*., 2014). With the study focus being plant response rather than crop performance, the genotype-within-plot was set as the experimental unit. Thus, when multiple plants were subsampled within a plot, they were considered as randomly selected representatives of any plant growing in the stand. Field observations and initial statistical tests identified non-linear soil gradients in each field trial, significantly affecting most traits measured. In 2015, the gradient followed a third order polynomial, whereas in 2016, the gradient followed a second order polynomial. For this reason, year-specific analyses were conducted for each trait, including position variables in models of the general form

$$\text{trait value} \sim X + c1 + c2 + c3 + (gtplot) + \varepsilon$$

where c1, c2 and c3 denote first, second and third polynomial orders of position in the field (c3 used in 2015 only), *gtplot* denotes the random effect of 'individuals of each genotype in a plot', and ε denotes the residual error. The main term X varied among models, depending on the plastic response to mixing being tested. For each plant functional trait, overall response to mixing was evaluated by setting *diversity level* as the main factor, thus testing for differences in trait values of plants (of any genotype) grown in mixtures and pure stands, respectively (i.e. *plastic response to mixing*). Cultivar differences in plastic response to mixing were evaluated by using cultivar, diversity level and their interaction as three main factors and testing for an interaction effect. The effect of cultivar pair (*mixture*) on plastic response was evaluated by using *mixture identity*, *diversity level* and their interaction as three main factors and testing for an interaction effect. *Neighbourhood effect* was evaluated by testing for this interaction effect on Salome only, being grown in mixture with each of the other four cultivars (*adaptive similarity*).

Release from competition-derived niche reinforcement was tested by assessing whether traits of two cultivars became more similar when they grew together in a mixture, as compared to the growth in a pure stand. As a measure of such *adaptive similarity*, we compared for each trait the average difference between cultivar pairs in their shared mixture ('Δ mix') and in their respective pure stand ('Δ pure'), using one-way ANOVA.

Overall plastic response was evaluated for all traits combined and for each of a number of trait groups, using the permutational multivariate analysis of variance through distance matrices included in the vegan package in R (Oksanen *et al.*, 2018). Trait groups comprised traits related to height/stretching (plant height, number of internodes, internode height and height per biomass), growth and vigor (number of tillers, leaf area, SLA, SPAD and biomass), N economy (leaf N content, grain N concentrations, N uptake efficiency, yield-specific N efficiency and NAE) and reproduction/yield (grain mass and harvest index).

Prior to all analyses, normality and homoscedasticity were determined for each trait by plotting the observed values against their residuals, and third-root or natural logarithm transformations were used to normalize variables with skewed distribution. Each trait was analysed at each measured time point (trial year and days after sowing).

Results

Overall effect of mixing

Mixing generally promoted final biomass and grain production of cultivars (Table 2). Cultivar mixtures had higher *harvest index* and higher *N concentration in the leaves* in 2015, and produced more *biomass* in 2016, reaching higher *grain mass* compared with pure stands. Plants in mixtures *developed* slower in both years and were *shorter* relative to pure stands early in the season (Figs. 1A, B and D). However, they had a higher *stretching rate* later in the growing season, ultimately reaching approximately the same height as plants in pure stands (Fig. 1C). The *N economy* of mixtures and pure stands was not significantly different in any year, and the same was true for all *leaf traits*.

Plastic response to mixing

Several growth related traits changed when individual cultivars were grown in mixtures compared to pure stand (Table 3). Plants responded to mixing with a slower *development*, shorter *plants*, and higher *stretching rates* (between 40 and 68 days after sowing and 52 and 68 days after sowing) with the *fourth internode* as the most plastic one.

Trait responses to mixing were found in 2015 for each of the five cultivars (Table 3). Salome responded to mixing with a slower *development of growth stages*. The most pronounced difference in plant development between cultivars in mixtures and pure stands was found for Salome, which was at BBCH 31 when grown together with Anakin, at BBCH 32 when grown together with Fairytale and Luhkas, and BBCH 41 when grown with Rosalina, while Salome in pure stand already was at BBCH 49. Fairytale and Anakin responded to mixing with *shorter plants* early in the season. Later in the season, Salome, Fairytale and Luhkas responded to mixing with a higher *stretching rate*. Anakin and Salome responded with lower *grain mass* relative to biomass (harvest index).

Mixture-specific trait responses were observed in 2015 (Table 4). Salome developed faster than in pure stand when grown together with Anakin (evaluated as BBCH at 13 July). Salome grown together with Rosalina had a higher *relative growth rate*, *RGR* (between 24 and 110 days after sowing) and a higher final *biomass production* (Table 4). Fairytale responded to mixing with smaller *plant height* and higher *late stretching rate*. Anakin responded with a greater *plant height* and a higher *harvest index* when grown together with Salome. In 2016, no mixture-specific cultivar responses were found, although all pair-wise mixtures varied significantly in overall.

Neighbourhood effect

Plastic responses depended on the neighbourhood of the focal plants. This was seen from the *number of tillers*, *RGR*, *SLA*, *biomass*, *grain mass*, and N accumulation efficiency, *NAE* in 2015 (Table 5). Some of the combined cultivars complemented each other by *NAE* and increased *grain mass* production, while this effect did not occur when grown with other cultivars. Salome had a higher *NAE* when grown with Rosalina than when growing with either of Fairytale or Anakin in 2015. Similarly, Salome grown with Rosalina produced more *grain-* and *vegetative biomass* than when grown with Fairytale, Anakin or Luhkas. Finally, Salome had a higher *relative stretching rate* (between 40 and 52 days after sowing) when grown with Luhkas than when grown with Fairytale. In 2016, no neighbor effects were observed, except for Salome grown with Rosalina, having a higher *chlorophyll content* in the first upper leaf than when grown with Anakin.

Adaptive similarity

Resource acquisition traits of mixed cultivars generally became more similar than traits of plants grown in pure stands (Table 6). For Salome and Luhkas mixed, such adaptive similarity was marked for *plant height* and *relative stretching rate* in 2015. Salome and Anakin were more similar in early *relative stretching rate* and N accumulation efficiency, *NAE* when they grew together in a mixture. In 2016, Salome and Anakin had a high adaptive similarity of *N content in their grains* and *yield-specific N efficiency* (Table 6). No adaptive similarity effects were found in the Salome-Fairytale and Salome-Rosalina mixtures. The overall trait difference in 2015 was smallest for the mixture Salome-Rosalina (Δ pure/Δ mix = 0.6) - indicating that they became most similar compared to growth in their respective pure stands, whereas Salome-Luhkas mixtures had the highest overall trait differences (Δ pure/Δ mix = 2.2). In 2016, the highest adaptive similarity was found for Salome-Luhkas (0.3) followed by Salome-Rosalina and Salome-Anakin (both 1.8) and Salome-Fairytale (2.2).

Plastic responses of trait groups

The effect of growing in a mixture was significant for *reproductive biomass* in 2016, which includes grain mass and harvest index (Table 7). In 2015, Plastic responses of cultivars were found for groups of traits related to *plant height*, *growth*, *N economy*, *reproductive biomass*, and *across all traits*. In 2016, this was true for the groups of traits related to *growth*, *N economy* and *reproductive biomass*. Plastic responses of cultivars grown in a mixture were found for *N economy* in 2016.

Discussion

Plastic response to mixing

We hypothesized that traits of individual cultivars tend to differ when plants are grown in a cultivar mixture as compared to a pure stand. We found that mixtures of barley cultivars developed slower early in the season relative to cultivars in pure stands. If plants in mixtures stay longer in the juvenile vegetative phase, they have more time for leaf- and spikelet primordia growth, which might partly explain the increased productivity often seen in mixtures. The transition from the vegetative to the adult phase is regulated among others by environmental cues (Bäurle & Dean, 2006). The altered environment in mixtures in terms of canopy structure could be one reason for a delayed transition. Plants grown in mixtures had a higher stretching rate relative to pure stands later in the season, reaching the same final height as plants in pure stand. In fact, height was the most plastic trait in these experiments, and significant changes in plant height were observed already at tillering (e.g. Fig. 1A). Luhkas, a fast-growing cultivar, was 23 % shorter in the beginning of the season when grown with Salome (Fig. 1A). This suggests a greater competition for light in the pure stand of Luhkas. In contrast, together with the shorter neighbour Salome, Luhkas did not have a strong competitor for light and did not need to invest in elongation. According to this anticipated shade avoidance mechanism, the short Salome was expected to extend in growth with the higher Luhkas in order to reach light, but interestingly Salome instead was 14 % shorter than when grown in pure stand (Fig. 1A), before increasing its relative stretching rate and reaching the same height as Luhkas at the last measurement (Figs. 1C and E). The results suggest that plants in cultivar mixtures invest less in shoots early in the season, possibly because they invest more into roots during this period to get sufficient resources for later accelerated growth in size. Previous reports showed that plant early response to the presence of neighbouring plants can result in altered biomass allocation to roots (Ninkovic, 2003; Falik *et al*., 2006). Faster establishment of roots into new nutrient patches can facilitate higher nutrient uptake and competitive ability relative to slower, less proliferating cultivars; but the relevance of these mechanisms for the specific mixtures compared here needs to be explored in further studies. Here, plastic changes in development, plant height and stretching rates (especially expressed in the fourth internode) affected productivity positively, confirming our first hypothesis. We compared plant growth in mixtures with that in pure stand using a replacement design, which means that any response of plants of a given cultivar is therefore due to the reduced density ("removed") of neighbouring individuals of the same cultivar, as much as it is the result of individuals of the other "added" cultivar.

Neighbourhood effect

We hypothesized that the plastic responses of plants grown in a mixture depend on the particular neighbourhood. This was tested by comparing the responses of Salome grown with all other cultivars separately. We found that plant traits associated with both resource acquisition, growth and fecundity changed plastically in response to neighbour cultivar, including RGR, SLA, grain mass, vegetative biomass, and NAE. Salome, for example, responded with increased N-uptake efficiency when grown with Rosalina, Fairytale and Luhkas, but not when grown with Anakin. Such plastic ability is genetically determined and we showed that plastic responses can vary upon different stimuli (neighbour cultivar). Our results highlight the importance of evaluating the effect of mixing on the performance of individual components in the mixture, rather than simply comparing the end product (yield) between cultivar mixture and pure stand (as for example in Kaut *et al.* (2009) and in Kiær *et al.* (2012)). Optimizing the performance of all component cultivars in a mixture seems to be a better way to enhance the mixed crop stand. To be able to find a mechanistic explanation for variation in mixing effects, all community components in a mixture have to be studied and not the mean of their responses. In our study, we analysed instead how the individual cultivars in a mixture respond to their neighbours in different traits through intermediated steps and developmental differences. This is the first study showing that the response to one individual cultivar can vary depending on the other cultivar in the mixture and that this plastic response can significantly influence productivity in mixtures. This finding can explain the inconsistent effects found in previous studies (e.g. Kiær *et al*., 2009; Reiss & Drinkwater, 2017), and the failure to find a general "mixture effect".

Adaptive similarity

We hypothesized that traits and properties of cultivars become more similar when the plants grow in a mixture. We found high adaptive similarity in stretching patterns (plant height and relative stretching rate) of two mixtures in 2015, while not in the other two. Cultivars Salome and Luhkas for example had a high adaptive similarity for plant height; they adapted their growth in plant height towards each other (Table 6). Plants can integrate the genetic identity of neighbours into appropriate adaptive responses by changing their allocation pattern, investing in height growth for not being shaded of taller neighbours (Fiorucci & Fankhauser, 2017) or root growth for acquisitioning similar amounts of nutrients (Craine & Dybzinski, 2013). Grady *et al.* (2016) found neighbour adaptation of trees and shrubs after replanting with genotypes from the same source locations and comparing with neighbours from different sources. Plant adaptation to the local environment has evolved through survival of the most adaptive genotypes in the wild. We have shown that this adaptive ability is retained in some cultivars even after hundreds of years of crop breeding. This observed reduction in niche differentiation suggests that the competitive release hypothesized to provide beneficial interactions in plant mixtures may eventually be smaller than anticipated.

Mixture effects on plastic responses

We hypothesized that plant trait diversity contributes to positive mixture effects on biomass and grain production. We found that the slower development of plants in cultivar mixtures did not affect their productivity. Cultivars grown in pure stands produced similar amounts of grain mass (Table 1). However, when two cultivars were grown together in a mixture – the lowest possible level of botanical diversity in agricultural fields - they tended to produce more grain and vegetative biomass compared to pure stands (not significant in 2015; Table 2). It seems that plants in pure stands were associated with faster maturity, and that they had less time for photosynthesis and grain filling compared with plants grown together with another cultivar. This is in line with the niche-partitioning hypothesis, which predicts that competition between close relatives will likely be stronger, due to niche overlap (File *et al.*, 2012). The results suggest that differences among the barley cultivars could result in a different exploitation and utilization of resources (complementary). Resource limitation is a key mechanism of increased productivity in diverse plant fields (Hooper *et al.*, 2005), which is in line with reports of greater mixture effects in low nutrient environments (Hooper & Vitousek, 1997). Increased beneficial results of cultivar mixtures on grain- and vegetative biomass production in harsh environmental conditions were found in other empirical studies (Reusch *et al.*, 2005; Tooker *et al.*, 2012; Kiær *et al.*, 2012) and in a meta-analysis (Reiss & Drinkwater, 2017). We want to emphasize, that these are row mixtures and that the effects might be higher in full mixtures.

Plastic responses of combined traits

Plastic responses of the combined grain mass and harvest index between mixtures and pure stands were higher in 2016 (Table 7), suggesting that cultivar mixtures are more productive in weather conditions more favourable for plant growth. This is in line with plastic responses of cultivars in 2015, 2016 and both years together, which differed in their reproduction traits. This suggests that the cultivars differed in their adaptation to the different weather conditions in these years, which is reflected by their growth and vigor together with their N economy.

Conclusions

This study makes an important contribution to understanding that plastic changes in plant traits are important for the interactions and eventual productivity of cultivar mixtures. The identification of differences in trait plastic responses of cultivars in different cultivar combinations highlights that these interactions are non-trivial, depending on the identity of the neighbour cultivar. Some cultivars complemented each other by N accumulation efficiency and yield-specific N efficiency, which resulted in increased grain and biomass production, while this effect did not occur when grown with other cultivar neighbours. The presented findings were based on row mixtures and even stronger plastic responses are expected in the full in-row mixtures, which are more commonly used. Derived effects of plastic responses in canopy traits on biotic interactions on plant diseases and insect herbivores are expected and were found before in some studies (e.g. Mundt, 2002; Ratnadass *et al.*, 2012). In summary, the results of this study provide a strong indication that trait plasticity might be a very important driver behind the variability in mixing effects. Intraspecific variation in plastic trait responses may affect niche partitioning and complementary among barley plants and their performance in multi-cultivar assemblages. Even a small increase in crop diversity could have a large impact on traits important for plant growth and nutrient acquisition, owing to specific properties of the component cultivars. Consequently, traits measured in pure stand may not be sufficient for the tailoring of efficient, stable and predictable cropping systems.

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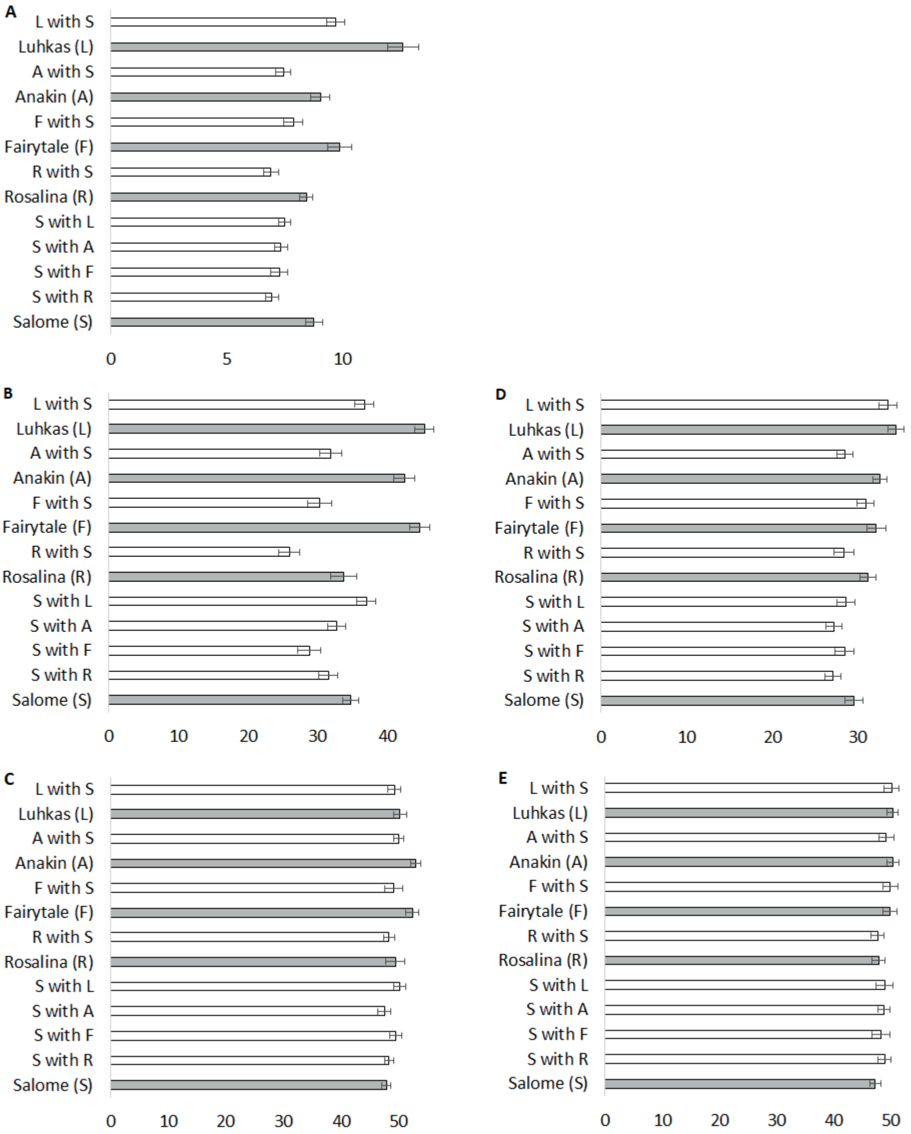

Figure 1. Plant height (cm) of cultivars grown in mixtures in all tested combinations (white bars) and in pure stands (grey bars), measured 40 (A), 52 (B) and 68 (C) days after sowing in 2015 and 47 (D) and 57 (E) days after sowing in 2016. Error bars indicate the sample standard error.

Table 1. The cultivars of spring barley used in this study.

Cultivar	Breeder	Pedigree	Type	Maturity (d)	Yield index*	Plant height (cm)[†]
Anakin	Sejet Plant Breeding, DK	Tumbler x Respons	Fodder	110	103	70
Fairytale	Sejet Plant Breeding, DK	Colston x (Recept x Power)	Fodder	110	101	71
Luhkas	R.A.G.T. Seeds Ltd, UK	Annabell x Prestige	Fodder	108	101	68
Rosalina	Sejet Plant Breeding, DK	Beatrix x Eskobar	Malt	109	99	68
Salome	Nordsaat Saatzucht GmbH, DE	Auriga x (Publican x Beatrix)	Malt	109	104	64

*percentage relative to a reference mixture of cultivars Prestige, Justina, Orthega and Gustav.

[†] multi-year-average (Larsson, Hagman, & Dryler 2013).

Table 2. Tests of the overall effect of mixing on plant functional traits, i.e. the plastic change of spring barley plants in response to growing in a mixture, and the proportional change (prop) as compared to pure stands. Significance of difference (* p<0.05; ** p<0.01; *** p<0.001) is marked in bold.

	2015			**2016**		
Plant trait	*df*	F	prop	*df*	F	prop
Growth stage[$]	72	**14.1*****	-6.9	76	**30.9*****	-3.0
No. of tillers[‡]	71	0.1	-0.8	73	1.7	3.7
RGR[†€]	74	2.8	4.0	75	2.2	3.5
SLA[#]	71	0.04	1.0	–	–	–
SPAD flag[#]	71	0.001	0.3	75	0.007	0.2
SPAD 2nd leaf[#]	71	1.2	2.9	75	0.8	1.7
Biomass[†]	71	2.6	-10.6	75	1.0	6.4
Biomass[#]	71	0.001	2.9	75	2.0	2.6
Biomass[€]	71	0.5	12.5	73	**9.5****	14.7
Height[‼]	72	**22.9*****	-31.6	75	**13.1*****	-11.1
Height[$]	72	**27.5*****	-24.5	73	0.2	-0.7
Height[#]	71	3.3	-2.8	–	–	–
Fourth internode[#]	72	6.8*	-23.3	70	0.5	-5.7
Stretching rate[‼$]	72	0.1	-0.7	75	**15.4*****	14.5
Stretching rate[‼#]	72	**21.4*****	11.3	–	–	–
Stretching rate[$#]	74	**33.0*****	97.1	–	–	–
N conc. leaves[†]	72	**5.1***	-21.6	71	0.7	-9.6
N conc. leaves[#]	71	1.1	57.4	71	0.3	0.6
N conc. grains[€]	71	1.5	-3.2	75	0.4	-4.2
N acc eff (NAE)[€]	71	0.2	1.4	71	0.1	5.9
N uptake eff (U_N)[€]	71	0.7	37.4	71	0.1	1.9
Yield-spe N eff ($EN_{,Y}$)[€]	72	1.1	-6.2	71	0.03	-2.5
Harvest index[€]	74	**10.3****	-5.0	75	2.3	-2.6
Grain mass[€]	71	0.05	3.0	73	**7.5****	10.6

[†] 24 days after sowing (das); [‡] 38 das; [‼] 40 das; [$] 52 das; [#] 68 das; [€] 110 das.

Table 3. To test for a general plastic response to mixing, we evaluated changes in the functional traits of each cultivar when grown in pairwise cultivar mixtures (with Salome evaluated across four mixtures with each of Anakin, Fairytale, Luhkas and Rosalina), as compared to their pure stands. Only significant trait responses are shown. For each plant trait, overall plastic response to mixing was evaluated by setting diversity level as the main factor, testing for differences in trait values between a genotype grown in mixtures and in pure stand. Showing degrees of freedom (df) and F-values; significance of difference (*P < 0.05; **P < 0.01; ***P < 0.001) is marked in bold.

Plant trait	***df***	**F**	**Anakin**	**Fairytale**	**Luhkas**	**Rosalina**	**Salome**
2015							
Growth stage[$]	64	0.7	-2.1	-2.0	-2.7	-1.3	**-5.4***
Height[$]	64	1.4	**-0.4***	**-0.5****	-0.2	-0.3	-0.1
Fourth internode[#]	64	2.5	**-1.9****	-0.3	0.2	-0.9	0.004
Stretching rate[!!#]	64	0.3	0.005	0.004	0.008	0.006	**0.008***
Stretching rate[$#]	66	2.1	0.07	**0.1*****	**0.09****	0.06	0.02
Harvest index[€]	66	0.3	**-0.04***	-0.03	-0.02	-0.02	**-0.03***

!! 40 days after sowing (das); $ 52 das; # 68 das; € 110 das.

Table 4. Tests of plastic responses in the functional traits of spring barley cultivars in specific pair-wise mixtures (plants grown with a specific neighbor cultivar) as compared to their respective pure stands, showing degrees of freedom (df) and F-values. Cultivars are Salome (S), Rosalina (R), Fairytale (F), Anakin (A) and Luhkas (L); the first letter indicating the cultivar of the focal plant and the second letter indicating the neighbor cultivar ('Salome mixed with Rosalina' etc.). Asterisks after F-values show test results for overall differences between cultivar treatments. Significance of difference (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) is marked in bold.

Plant trait	***df***	**F**	**SR**	**SF**	**SA**	**SL**	**RS**	**FS**	**AS**	**LS**
2015										
Growth. stage[$]	61	**2.1***	4.7	6.0	**6.6***	4.2	1.3	2.0	2.1	2.6
No. of tillers[‡]	60	**3.6*****	-0.3	0.04	0.3	0.002	-0.3	0.1	0.08	0.2
RGR[†€]	63	**2.3***	**-0.005***	-0.002	-0.001	-0.001	-0.003	0.001	-0.002	0.001
SLA[#]	60	**2.8****	2.6	-8.2	-7.3	-11.1	-10.2	17.4	3.8	6.4
SPAD flag[#]	60	**3.6*****	-1.2	1.6	2.3	-2.0	2.1	0.2	-0.2	1.0
SPAD 2nd leaf[#]	60	**3.5*****	-3.5	-1.3	-0.3	-2.0	-0.5	-1.3	0.3	0.7
Biomass[€]	60	**3.4*****	**-0.1****	-0.009	0.04	-0.01	-0.1	0.01	-0.03	0.04
Height[!!]	61	**4.8*****	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.2
Height[$]	61	**5.8*****	0.2	0.3	0.1	-0.03	0.3	**0.5****	**0.4***	0.2
Fourth internode[#]	61	**2.1***	0.04	-0.6	0.1	0.3	0.9	0.3	**1..9****	-0.2
Stretching rate[!!$]	61	**2.3***	-0.01	0.002	-0.01	-0.02	0.006	0.02	0.008	-0.003
Stretching rate[!!#]	61	**5.6*****	**-0.01***	0.009	-0.007	-0.008	-0.006	-0.004	-0.005	-0.008
Stretching rate[$#]	63	**7.2*****	-0.03	-0.05	-0.01	0.004	-0.06	**-0.1*****	-0.07	**-0.09****
N conc. grains[€]	60	**3.2****	-0.1	0.01	0.07	0.002	-0.1	0.04	0.003	0.06
N acc eff (NAE) [€]	60	**3.5*****	-0.3	0.1	0.3	0.1	-0.3	-0.04	-0.05	0.3
N uptake eff (U_N) [€]	60	**2.8****	-0.3	-0.1	0.06	-0.08	-0.2	-0.09	-0.08	0.1
Harvest index[€]	63	**5.6*****	0.03	0.03	0.03	0.02	0.02	0.03	**0.04***	0.02
Grain mass[€]	60	**3.2****	-0.1	0.01	0.07	0.002	-0.1	0.04	0.003	0.06
2016										
SPAD 2nd leaf[#]	64	**2.0***	-1.8	0.2	1.5	0.5	1.1	-1.0	-0.7	-1.1

Plant trait	*df*	F	SR	SF	SA	SL	RS	FS	AS	LS
Biomass€	62	**2.1***	-0.03	-0.03	-0.03	0.02	-0.04	-0.05	-0.07	-0.009
Height‼	64	**3.9*****	0.05	0.008	0.06	0.002	0.1	-0.03	0.1	0.07
Fourth internode#	59	**2.2***	0.007	-0.3	-0.6	-0.7	-0.008	0.4	0.1	0.3
Stretching rate‼$	64	**2.9****	-0.009	-0.005	-0.009	-0.005	-0.01	-0.001	-0.01	-0.004
N conc. grains€	64	**2.8****	-0.1	0.1	0.2	-0.04	0.1	0.05	-0.2	-0.05
N uptake eff (U_N) €	60	**6.2*****	-0.1	-0.06	0.02	-0.02	-0.2	-0.04	0.08	0.004
Yield-specific N eff ($E_{N,y}$) €	60	**3.4*****	0.01	-0.07	-0.1	-0.03	-0.01	-0.07	0.2	0.02
Harvest index€	64	**7.8*****	0.01	0.008	0.01	0.01	0.02	-0.006	0.02	0.01
Grain mass€	62	2.0*	-0.02	-0.02	-0.03	0.03	-0.03	-0.06	-0.06	-0.001

† 24 days after sowing (das); ‡ 38 das; ‼ 40 das; $ 52 das; # 68 das; € 110 das.

Table 5. Tests of neighbor effect, i.e. whether plastic response in the functional traits of Salome (S) plants when growing in a mixture (relative to the pure stand) depends on the neighbor genotype (Rosalina (R), Fairytale (F), Anakin (A) respectively Luhkas (L)). Differences in plastic response of Salome were evaluated by using neighbor cultivar, diversity level and their interactions as three main factors and testing for an interaction effect. Asterisks for F-values shows whether the response depended on neighbor overall, and the other columns show pairwise comparisons between neighbor treatments. Significance of difference (**P* < 0.05; ***P* < 0.01; ****P* < 0.001) is marked in bold.

Plant trait	*df*	F	SR-SF	SR-SA	SR-SL	SF-SA	SF-SL	SA-SL
2015								
No. of tillers‡	17	**4.7***	0.3	**0.6****	0.3	0.2	-0.03	-0.3
RGR†€	20	**3.2***	0.003	0.003	0.004	0.001	0.001	0.001
SLA#	17	**3.8***	-10.4	-10.3	-13.8	0.04	-3.5	-3.5
Biomass€	17	**6.5****	**0.1***	**0.2*****	**0.1***	0.06	0.003	-0.05
Stretching rate‼$	18	2.9	0.02	0.001	-0.006	-0.01	**-0.02***	-0.007
N acc eff (NAE) €	17	**4.4***	**0.5***	**0.7****	**0.5***	0.2	-0.02	-0.2
Grain mass€	17	**5.1***	**0.1***	**0.2****	0.1	0.07	-0.006	-0.07
2016								
SPAD 2nd leaf#	19	3.3	1.9	**3.3***	2.2	1.4	0.3	-1.1

† 24 days after sowing (das); ‡ 38 das; ‼ 40 das; $ 52 das; # 68 das; € 110 das.

Table 6. Ratios signifying whether cultivar traits became more similar when they grew in a mixture ('Δ mix' in combinations of Salome (S), Rosalina (R), Fairytale (F), Anakin (A) and Luhkas (L)), as compared to the trait difference between pure cultures ('Δ pure'). Ratios significantly different from 1 (**P* < 0.05; ***P* < 0.01; ****P* < 0.001) are marked in bold.

	SR		**SF**		**SA**		**SL**	
Plant trait	Δ pure	Δ pure / Δ mix	Δ pure	Δ pure / Δ mix	Δ pure	Δ pure / Δ mix	Δ pure	Δ pure / Δ mix
2015								
Height$	1.0	1.1	0.92	1.1	0.94	1.1	0.91	**1.1***
Stretching rate‼$	0.98	1.2	0.90	1.1	0.90	**1.2***	1.1	1,1
Stretching rate$#	0.97	0.90	1.3	0.78	1.2	0.827	1.6	**0.65***
N acc eff (NAE) €	0.98	0.98	1.0	0.97	1.1	**0.88***	0.98	1.0
2016								
N conc. grains€	1.0	0.91	0.96	1.0	0.80	**1.3****	1.0	1.0
Yield-specific N eff ($E_{N,y}$) €	1.0	0.97	1.0	1.0	0.87	**1.2***	1.0	0.61

‼ 40 days after sowing (das); $ 52 das; # 68 das; € 110 das.

Table 7. Plastic responses of combined functional traits assessed in 2015, 2016 and both years combined. For definitions of trait groups, see text. Significance of difference between cultivar in mixtures and pure stands (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) are marked in bold.

		2015	**2016**	**2015 + 2016**
Source of variation	*df*	F-value	F-value	F-value
			All traits	
Diversity level	1	0.22	0.23	0.17
Cultivar	4	**3.99****	1.67	1.40
Diversity level*Cultivar	4	0.79	0.53	0.33
			Height/stretching	
Diversity level	1	2.66	0.14	1.48
Cultivar	4	**1.98***	1.15	1.82
Diversity level*Cultivar	4	0.80	0.28	0.54
			Growth and vigor	
Diversity level	1	0.18	0.23	0.098
Cultivar	4	**4.06*****	**2.17***	1.57
Diversity level*Cultivar	4	0.74	0.47	0.068
			N economy	
Diversity level	1	0.55	0.30	0.14
Cultivar	4	**3.86*****	**2.36****	1.80
Diversity level*Cultivar	4	0.73	**3.27****	1.24
			Reproduction/yield	
Diversity level	1	0.87	**4.25***	1.52
Cultivar	4	**2.74***	**3.15****	**3.28***
Diversity level*Cultivar	4	1.28	0.26	0.39

IV

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Pest suppression in cultivar mixtures is influenced by neighbor-specific plant–plant communication

IRIS DAHLIN,[1,3] DIANA RUBENE,[2] ROBERT GLINWOOD,[1] AND VELEMIR NINKOVIC[2]

[1]*Department of Crop Production Ecology, Swedish University of Agricultural Sciences, P.O. Box 7043, SE-75007 Uppsala Sweden*
[2]*Department of Ecology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-75007 Uppsala Sweden*

Abstract. Increased plant genotypic diversity in crop fields can promote ecosystem services including pest control, but understanding of mechanisms behind herbivore population responses to cultivar mixtures is limited. We studied aphid settling on barley plants exposed to volatiles from different cultivars, aphid population development in monocultures and two-cultivar mixtures, and differences in volatile composition between studied cultivars. Aphid responses to one cultivar in a mixture were neighbor-specific and this was more important for pest suppression than the overall mixture effect, aphid colonization patterns, or natural enemy abundance. Aphid populations decreased most in a mixture where both cultivars showed a reduced aphid–plant acceptance after reciprocal volatile exposure in the laboratory, and reduced population growth compared to monocultures in the field. Our findings suggest that herbivore population responses to crop genotypic diversity can depend on plant–plant volatile interactions, which can lead to changes in herbivore response to individual cultivars in a mixture, resulting in slower population growth. The impact of plant–plant interaction through volatiles on associated herbivore species is rarely considered, but improved understanding of these mechanisms would advance our understanding of the ecological consequences of biodiversity and guide development of sustainable agricultural practices. Combining cultivars in mixtures based on how they interact with each other is a promising strategy for sustainable pest management.

Key words: aphid; botanical diversity; cultivar mixtures; functionality; genotype; herbivore suppression; intraspecific plant diversity; pest management; plant signal substances; plant–herbivore interactions; plant–plant communication; volatile organic compound.

INTRODUCTION

Plant species diversity promotes stability, productivity, and resilience in natural and agricultural ecosystems (de Mazancourt et al. 2013, Prieto et al. 2015, Isbell et al. 2017), whereas loss of diversity can lead to degradation of these services (Oliver et al. 2015). Though less studied, the effects of genotypic diversity on ecosystem function may be similar to those of species diversity (Hughes et al. 2008, Tooker and Frank 2012). Previous studies have shown effects of plant genotypic diversity on productivity (Cook-Patton et al. 2011, Reiss and Drinkwater 2017), herbivore spatial distribution (Utsumi et al. 2011, Zytynska et al. 2014) and dynamics (Underwood 2009), arthropod richness (Crutsinger et al. 2006), herbivore and pathogen damage (Peacock et al. 2001) and disease management (Mundt 2002).

Increased genotypic plant diversity in crop fields could potentially improve system resilience (Bullock et al. 2017), and promote ecosystem services such as pest control, both of which are major challenges for agricultural sustainability (Tilman et al. 2002), but empirical evidence for its effectiveness is weak. Laboratory studies of cultivar mixtures have shown improved insect pest suppression in certain cultivar combinations (Shoffner and Tooker 2013, Grettenberger and Tooker 2017), but there are few evaluations of pest suppression in the field. Previous studies have shown no herbivore response to genotypic diversity (Hambäck et al. 2010), or a positive or negative mixture effect on mobile herbivores, likely caused by variation in colonization rates and increased movement among cultivars (Peacock et al. 2001, Underwood 2009, Utsumi et al. 2011). However, understanding of the mechanisms behind effects of cultivar mixtures is hindered because most studies only assess differences between monocultures and mixtures, without measuring herbivore response to individual mixture components (but see Utsumi et al. 2011). In fact, cultivar or species mixtures per se are not guaranteed to reduce pest impact (Tooker and Frank 2012) and insect responses to agricultural diversity are system dependent (Barbosa et al. 2009, Ratnadass et al. 2012). Therefore, knowledge of the processes underlying differential pest responses is needed to understand the ecological consequences of biodiversity and to develop sustainable agricultural practices based on botanical diversity.

Specific plant associations can positively or negatively affect a plant’s vulnerability to herbivory (associational susceptibility vs. associational resistance; Barbosa et al. 2009), suggesting that the “right kind of diversity” is needed to obtain the desired effect. Among mechanisms proposed to explain how increased botanical diversity reduces pathogen and pest populations is the dilution effect, which implies that if plants differ in resistance levels, an increase in distance between susceptible plants reduces the rate of pest spread by increasing herbivore search time (Root 1973, Malézieux et al. 2009, Hambäck et al. 2014). The natural enemy hypothesis predicts pest suppression through increased abundance of natural enemies due to larger variety of food resources (Elton 1958, Root 1973, Cook-Patton et al. 2011).

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[3] E-mail: Iris.dahlin@slu.se

The impact of one potentially important mechanism has received considerably less attention: chemical communication between neighboring plants leading to changes in plant physiology that affect insects (reviewed by Ninkovic et al. 2016). Plant volatile signaling of pest and pathogen attack is well described (Engelberth et al. 2004), but plants also use volatile organic compounds (VOCs) to detect the presence, absence, and identity of neighboring plants (Callaway 2002). Both damaged and undamaged plants emit VOCs that can induce growth responses in receiver plants, which in turn might affect other organisms that use the plant as a host (Dicke 1994, Ninkovic et al. 2013). Better understanding of volatile plant–plant interactions and their effect on herbivores may explain apparently inconsistent effects of genetic diversity and would enable more accurate predictions of herbivore suppression in cultivar mixtures.

We used a model system of barley *Hordeum vulgare* L. and the bird cherry-oat aphid *Rhopalosiphum padi* L. to examine plant–plant communication and plant–insect interactions in cultivar mixtures, and to explore the impact of genotypic diversity on aphid–plant acceptance and population development. We combined laboratory experiments, manipulation of genotypic diversity in the field and VOC analyses of individual cultivars to investigate variation in aphid responses to cultivar mixtures and the association between these responses and cultivar VOC profiles. Earlier studies suggest that chemical interactions between plants may be important drivers of aphid responses to specific cultivar mixtures (Ninkovic et al. 2002). Assuming that plant interactions are the main cause of inconsistency in pest responses, we hypothesized that mixing two cultivars would lead to one of three outcomes: (1) cultivars do not react to each other and aphid response is similar to monocultures, (2) one of the cultivars induces physiological responses in the other cultivar that affect aphid–plant acceptance and their population development, or (3) both cultivars respond to each other with a combined effect on aphids. Aphids can cause significant yield and economic loss to grain crops globally (Valenzuela and Hoffmann 2015) and conventional control methods cause pesticide pollution and insecticide resistance (Pimentel et al. 1992). This study highlights the potential of cultivar mixtures in reducing these negative impacts in agriculture.

Materials and Methods

Laboratory experiment

Spring barley cultivars Salome, Fairytale, Rosalina, Anakin, and Luhkas (obtained from Scandinavian Seed AB, Lidköping, Sweden) were used (Appendix S1: Table S1). None of the cultivars were bred for aphid resistance. Six plants were grown together in a plastic pot (8.5 × 7 cm) in potting soil (Hasselfors Garden, Örebro, Sweden), in a greenhouse at 18–22°C with a light regime of 16 h : 8 h light : dark and used for exposure experiments eight days after sowing. Test aphids were taken from a multi-clonal population of bird cherry-oat aphid *R. padi* reared on oats in a separate greenhouse chamber under the same conditions as the plants.

One barley cultivar was exposed to VOCs from another cultivar in a series of transparent two-chamber cages consisting of an inducing and a responding chamber (Ninkovic et al. 2002). Air passed over one cultivar in the inducing chamber through an opening into the responding chamber containing another cultivar before being vented from the exposure room. For control plants, the inducing chamber was empty. After five days of exposure, five randomly chosen responding plants were used for aphid acceptance tests. Barley plants at the same phenological stage as during aphid colonization in the field were used for aphid acceptance tests, to simulate the establishment phase. Aphid–plant acceptance was measured with a no-choice plant settling test (Ninkovic et al. 2002). A 50-mL polystyrene tube was placed over the second leaf of each of the tested plants per pot containing 10 randomly chosen wingless aphids of the third and fourth instar. In total, 20 plants (replicates) per each treatment were tested. After 2 h, the number of aphids settled on each leaf was recorded and expressed as a proportion of the 10 introduced aphids.

Field experiment

The field experiment was conducted at Lövsta field station, Uppsala, Sweden (59°52′ N, 17°48′ E). Barley was sown in the beginning of May at 400 viable seeds/m^2 with a row spacing of 12.5 cm. Nine treatment plots (3 × 9 m) were randomly placed in each of six blocks in a conventional randomized block design with a distance of 1 m between plots (without any vegetation), each of these representing a replicate. The same cultivars as for laboratory experiments were grown in plots as monocultures or in two-cultivar mixtures in alternate rows, enabling cultivar identification of individual plants. The cultivar Salome was chosen as a component in all mixtures because it affected aphid acceptance on other cultivars in the laboratory experiments.

To measure aphid immigration to the field, yellow water-traps (26 cm diameter; Flora Modéle Déposé, Ringot, France) containing water and a drop of detergent were, in four blocks, placed in the center of each plot. Traps were adjusted weekly to be always at canopy height and trapped aphids were sampled weekly.

Aphid abundance per plot was determined by recording the number of aphids on plants along three randomly chosen 1-m transects in each plot for each cultivar and summing the three values (Ninkovic et al. 2003). Observations were made twice weekly for four consecutive weeks, from 1 June to 1 July.

The abundance of predatory arthropods, such as ground-dwelling carabids and spiders, was determined by weekly sampling of one pit-fall trap per plot. Estimates of seven-spot ladybird *Coccinella septempunctata* L. occurrence were made simultaneously by two observers, one on each half of the plot, to avoid recording the same individual twice (Ninkovic et al. 2011). Observations were made once per week during June.

Volatile organic compounds

Plant VOCs were collected by dynamic headspacing (Appendix S2). Air was drawn over a molecular absorbent from which VOCs were extracted using solvent. Samples were analyzed by gas chromatography/mass spectrometry.

Compounds were identified by matching with commercially available libraries and authentic chemical standards, and quantified using response curves for authentic standards (Glinwood et al. 2011).

Statistical analyses

Aphid–plant acceptance.—Differences in aphid–plant acceptance between cultivars exposed to clean air and cultivars exposed to VOCs from other cultivars were analyzed with generalized linear mixed models (GLM) with binomial error distribution in lme4 (Bates et al. 2015) in R (R Core Team 2016), with the proportion of settled aphids out of 10 introduced aphids as a replicate. We ran one model for each receiving cultivar (S, A, R, F, and L), where the proportion of aphids settled on control plants was used as reference. Emitting cultivars were used as explanatory factors and pot and block as random factors to control for spatial arrangement of plants.

Aphid immigration.—GLM with Poisson error distribution in R, lme4, were used to assess differences in aphid immigration between monocultures and mixtures. The total number of immigrating winged *R. padi* per plot during the first two weeks of colonization (1–14 June) was used as the response, cultivar (or mixture) identity as fixed explanatory factor, block as a random factor, and an additional observation-level random factor to control for overdispersion.

Aphid population response.—Aphid population increase over time was analyzed using a Bayesian framework by fitting a logistic growth model to cumulative population data. We estimated differences in aphid population growth on cultivars grown in monocultures and in mixtures. To obtain a straightforward estimate of total population size, we calculated cumulative aphid abundance per plot (sum of three 1-m rows) for 10 survey days between 1 June and 1 July, for each plot and for each cultivar within mixed plots, resulting in 17 replicated cultivar combinations (listed in Table 2). Plot values for mixed plots were calculated as averages of the individual cultivars. One block was excluded due to low aphid abundance and poor plant development caused by heterogeneous soil conditions at the field edge.

To estimate population sizes, we fitted a logistic growth model to the cumulative aphid population data where the number of aphids was assumed to follow a lognormal distribution. The model was defined as follows:

$$\text{aphids}_i \sim \text{Lognormal}(\alpha_i, \tau)$$

$$\alpha_i = \log(\mu_i) - \sigma^2/2$$

$$\mu_i = \alpha_{\text{block}\cdot j} + V_{\max_s} \times \text{plogis}(c \times (\text{day}_i - h_p))$$

$$\alpha_{\text{block}\cdot j} \sim \text{Normal}(\mu_{\text{block}\cdot j}, \sigma_{\text{block}})$$

$$V_{\max\cdot s} \sim \text{Normal}(\mu_{v_{\max\cdot s}}, \sigma_{v_{\max}})$$

$$h_p \sim \text{Normal}(\mu_{h\cdot p}, \sigma_h)$$

where μ is the mean number of aphids per plot, $V_{\max}$ is the maximum (final) aphid population size, h is halftime (in d) when one-half of the total population size is reached, c describes the steepness of the growth curve, plogis is the logistic distribution function, and α_{block} is the block effect. $V_{\max}$ and h values were allowed to differ for each cultivar/mixture using cultivar-specific group effects drawn from normal distributions, while a single c was estimated for all cultivars/mixtures (mean = 0.35, SD = 8.97×10^{-3}). We estimated an individual $V_{\max}$ and h value for each of the 17 combinations and calculated the differences between these with 95% Bayesian credible intervals (CI). In order to determine if the effect of cultivar mixtures was additive or interactive, we also estimated the expected $V_{\max}$ with 95% CI for the mixtures as the average of two monocultures.

The model was implemented in JAGS software v. 4.2.0. (Plummer 2003) called from R using the rjags package (Plummer 2016). Weakly informative priors were used, not allowing parameters to take values outside the range of data, either normally distributed truncated at zero or uniformly distributed. We ran three Markov chains with 200,000 iterations after a 200,000 burn-in, thinned by five. The full model code with priors and initial parameter values is reported in Appendix Data S1. Model convergence was assessed by visual inspection of the trace plots and with the Gelman-Rubin convergence statistic (Gelman et al. 2004). Model fit was evaluated by simulating a data set under the model assumptions and comparing the sums of squares of simulated data to the observed data using posterior predictive checks and Bayesian P values (Gelman et al. 2004). We also compared our model with a simpler model that does not include cultivar/mixture specific effects for $V_{\max}$ and h using posterior predictive loss (Gelfand and Gosh 1998, Hooten and Hobbs 2015). The model showed a good fit with sum of squares $P = 0.34$ ($P = 0.5$ represents a perfect fit and $0.1 < P < 0.9$ an acceptable fit), and the full model had a lower posterior predictive loss compared to the simple model, indicating that cultivar/mixture specific effects were important for explaining aphid population growth (full, model fit component $[G] = 6.5 \times 10^8$, penalty component $[P] = 6.3 \times 10^8$, posterior predictive loss $[D]_{\infty,\text{sel}} = 1.3 \times 10^9$, vs. simple, $G = 7.9 \times 10^8$, $P = 8 \times 10^8$, $D_{\infty,\text{sel}} = 1.6 \times 10^9$).

Previous simulation studies have suggested that slow population increase early in the season gives a lower probability of reaching outbreak levels later in the season (Wiktelius and Pettersson 1985). To test whether final population size depended on the population growth earlier in the season, we analyzed the relationship between $V_{\max}$ and h obtained from the model in a GLM with gamma error distribution with a "log" link, in lme4.

Predators.—Effect of predator abundance (activity density) on aphid population size was analyzed with GLM with Poisson errors in lme4. Cumulative aphid population size on 1 July was used as response, the total abundance of ladybirds, ground-dwelling carabids, and spiders as a fixed explanatory effect, block as a random effect, and an additional observation-level random factor to control for overdispersion. We also analyzed the effect of cultivar mixture and aphid abundance on predator abundance using the same model structure as above.

Volatile organic compounds.—Dissimilarity in chemical composition between cultivar types was analyzed using non-metric multidimensional scaling (NMDS) in the R package vegan (Oksanen et al. 2017), using two dimensions ($k = 2$) and the Bray-Curtis index as a dissimilarity metric. Amounts of chemical components were rescaled to frequencies in the data, to remove the effect of differences in overall amount between individual replicates, e.g., pots of plants, and corrected for differences in biomass. Cultivar type was fitted to the ordination using the envfit function, which estimates a goodness-of-fit statistic (r^2) and assesses its significance using random permutations of the data.

In addition to comparison of odor profiles (above), we analyzed differences in amount (ng/g) and frequency of individual chemical components between cultivars. We used GLM with gamma errors with a "log" link in lme4 and square-root-transformed data as the response. Frequencies were analyzed using binomial errors. Salome was used as the reference category in all models, as it emitted the highest number and amount of VOCs; additional models with other cultivars as reference were run when needed to determine all pairwise differences. Based on dissimilarities in cultivar odor profiles and observed aphid-response patterns, we expected VOCs that could be involved in plant–plant interactions to be more abundant in Salome and Fairytale compared to Luhkas, Anakin, and Rosalina.

RESULTS

Aphid–plant acceptance

The exposure of barley plants to VOCs from a different cultivar resulted in significantly reduced aphid acceptance in six out of 20 cultivar combinations. The VOC exposure effects are both receiver and emitter specific. Two cultivars induced strong effects as emitters (Fairytale and Salome), while Anakin was a highly responsive receiver (Table 1; model output in Appendix S3: Table S1).

Aphid immigration and population response

There was no difference in aphid immigration between cultivar mixtures and monocultures (Appendix S4: Table S1).

The estimated aphid population size on different cultivars ($V_{\max}$) ranged from 3202 ± 283 (mean ± SE) to 4846 ± 327 aphids per plot, with the lowest numbers observed on Salome and Fairytale when grown in mixture (Table 2). The observed population size in the Salome–Fairytale mixture was significantly lower (27%) than expected aphid populations of these cultivars grown in monoculture (Fig. 1A). All differences between individual cultivars in pure and mixed stands were positive, with a confidence level of 74–100%, i.e., aphid populations were smaller when the cultivars were grown in mixtures compared to pure stands (Fig. 1).

TABLE 1. The ratio of mean number of aphids that accepted plants treated with VOCs to the mean number of aphids that accepted control plants.

	Emitter				
Receiver	Anakin	Fairytale	Luhkas	Rosalina	Salome
Anakin		0.85**	0.96	0.87*	0.80***
Fairytale	1.03		0.95	0.91	0.84**
Luhkas	0.94	1.04		0.95	0.87**
Rosalina	0.99	1.00	1.01		0.97
Salome	0.93	0.85**	1.12*	0.96	

Notes: Reduced acceptance gives a ratio of <1 while a ratio of 1 indicates no difference between treated and control plants. Significant differences in estimates are indicated by asterisk (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Differences in aphid population development early in the season were observed on the individual cultivars Salome and Fairytale grown in mixture compared to these cultivars in monocultures and for Salome grown with Anakin, while the other cultivars showed a similar population development on monocultures and mixtures. Aphid populations reached one-half of their size by day 18–21, and halftime (h) was a relevant factor explaining the final population size (estimate = −0.07, SE = 0.03, $t = -2.07$, $P = 0.055$), based on the difference in the Akaike information criterion (AIC) between the model including h and an intercept-only model (ΔAIC = 2.09).

Predator abundance

Predator abundance was not related to aphid population size (Appendix S5: Table S1). Neither cultivar mixture nor aphid abundance were relevant factors for explaining variation in predator abundance (Appendix S6: Table S1 and S7: Table S1).

Volatile organic compounds

Cultivars differed significantly in their volatile composition (NMDS with envfit, $k = 2$, stress = 0.13, $r^2_{\text{cultivar}} = 0.67$, $P = 0.001$). The odor profiles of Salome and Luhkas, and those of Anakin and Rosalina were compositionally similar, and certain compound groups were more closely associated with some cultivars, such as terpenoids with Luhkas and alkanes with Anakin (Fig. 2). Several components were found in significantly higher concentrations in Salome compared to other cultivars (Appendix S8: Table S1).

DISCUSSION

We show that herbivore populations develop differently in genotype mixtures, possibly induced by volatile interactions of the individual genotypes combined in a mixture, making these plants less susceptible to aphids. Volatile interactions between undamaged plants of certain cultivars in the laboratory lead to significantly reduced aphid–plant acceptance, and consequently, the same cultivars had also the lowest aphid population sizes when they were grown together in the field. Additionally, the interacting cultivars differed in their volatile profiles and amount of specific volatile compounds. This suggests that volatile interactions between cultivars might drive induced resistance, explaining why certain plant genotype combinations have stronger effects on pests than others do. We show that significant reduction of pest populations in the field can be achieved at the lowest level of diversity, by combining two genotypes when interacting with each other through VOCs, suggesting that volatile chemical

TABLE 2. Total aphid population sizes ($V_{\max}$) and halftime to population maximum (h) for cultivars and mixtures estimated by the population growth model.

	Aphid population size ($V_{\max}$)				Halftime, h (d)		
Treatment	Mean	2.5% CI	97.5% CI	Confidence	Mean	2.5% CI	97.5% CI
Monoculture	4,507	4,202	4,825				
Mixed	3,984	3,677	4,313				
Monocultures							
Salome (S)	4,846	4,232	5,529		19.6	18.7	20.4
Rosalina (R)	4,149	3,583	4,786		18.9	18.0	19.8
Fairytale (F)	4,086	3,511	4,724		18.3	17.4	19.3
Anakin (A)	4,713	4,098	5,375		19.3	18.4	20.1
Luhkas (L)	4,753	4,155	5,410		17.9	17.0	18.8
Mixtures							
SR	4,161	3,590	4,820		20.1	19.2	20.9
SF	3,277	2,762	3,892		20.3	19.3	21.3
SA	4,199	3,623	4,870		20.4	19.5	21.2
SL	4,284	3,710	4,924		19.9	19.1	20.7
Expected population sizes in mixtures							
S + R exp	4,495	4,055	4,961				
S + F exp	4,459	4,023	4,926				
S + A exp	4,784	4,331	5,263				
S + L exp	4,798	4,345	5,272				
Individual cultivars							
S in SR	4,412	3,786	5,083	0.83	20.3	19.5	21.2
R in SR	3,845	3,284	4,494	0.75	19.9	19.0	20.8
S in SF	3,328	2,792	3,937	1	20.6	19.6	21.5
F in SF	3,202	2,683	3,796	0.98	20.2	19.2	21.2
S in SA	3,947	3,354	4,619	0.97	20.9	20.0	21.8
A in SA	4,415	3,799	5,097	0.74	20.0	19.1	20.9
S in SL	4,156	3,579	4,814	0.94	20.4	19.6	21.3
L in SL	4,362	3,779	5,035	0.82	19.5	18.6	20.3

Notes: Cultivars written as, e.g., "S in SR" refer to individual cultivars in mixed plots (i.e. Salome when grown with Rosalina), while "SR" refers to the average value of the mixed plot, and "S + R exp" is the expected mixed plot value, calculated as the average of population sizes in monocultures. "Monoculture" and "Mixed" refer to estimated mean population size for pure and mixed cultivar plots, respectively. Values are means with upper and lower Bayesian credible intervals. Model-estimated confidence level (i.e., the proportion of posterior probability distribution above zero) is shown for the differences between cultivars in pure and mixed plots.

interactions between plants play an important role in mediating plant–pest interactions.

Aphid–plant acceptance

We found a significant reduction in aphid–plant acceptance for certain cultivars after exposure to another specific cultivar in the laboratory (Table 1). Such reductions after plant exposure in the laboratory were also found in plant acceptance tests in the field, when the cultivar was grown in the same combination in a mixture (Ninkovic et al. 2002). Plant acceptance is a key factor in the progress of an aphid infestation (Pettersson et al. 2007) and is correlated with subsequent aphid growth rate (Ninkovic et al. 2009, Dahlin and Ninkovic 2013). The exposure effect is VOC emitter and receiver specific. Cultivar Salome has a strong effect as an emitter and was therefore tested in the field in combination with the other cultivars. The combination Salome and Fairytale resulted in significantly reduced aphid population growth in the mixture, with lower aphid numbers on both cultivars than in pure stands. This fits well with the results from the laboratory experiment, showing that both cultivars function as emitter and as receiver in this combination. Combinations where a receiver or non-receiver cultivar was grown with the emitter Salome did not result in significantly reduced aphid population growth, indicating that both cultivars need to respond as emitter and receiver to have an effect under field conditions.

Plant–plant communication

Plants are rooted and thus unable to escape unfavourable conditions, competitors or attackers. However, plants are not defenceless; they have evolved to detect and respond to VOCs from other plants in order to survive. They can detect volatile cues from herbivore- or pathogen-attacked neighbors (Engelberth et al. 2004) and these cues can regulate specific and effective biochemical defense pathways (Erb 2018). Plants also use volatile cues to detect the presence and identity of other plants (Callaway 2002). For example, parasitic plants use VOCs to locate their host plants (Mescher et al. 2006). Recent research has shown that neighbor detection in undamaged plants via volatile cues can be specific and may be a mechanism by which plants detect and prepare for future competition (Ninkovic et al. 2016). Plant growth and physiological changes in response to volatile cues can affect other organisms that use the plant as a host (Dicke 1994, Ninkovic et al. 2013). Aphids are very

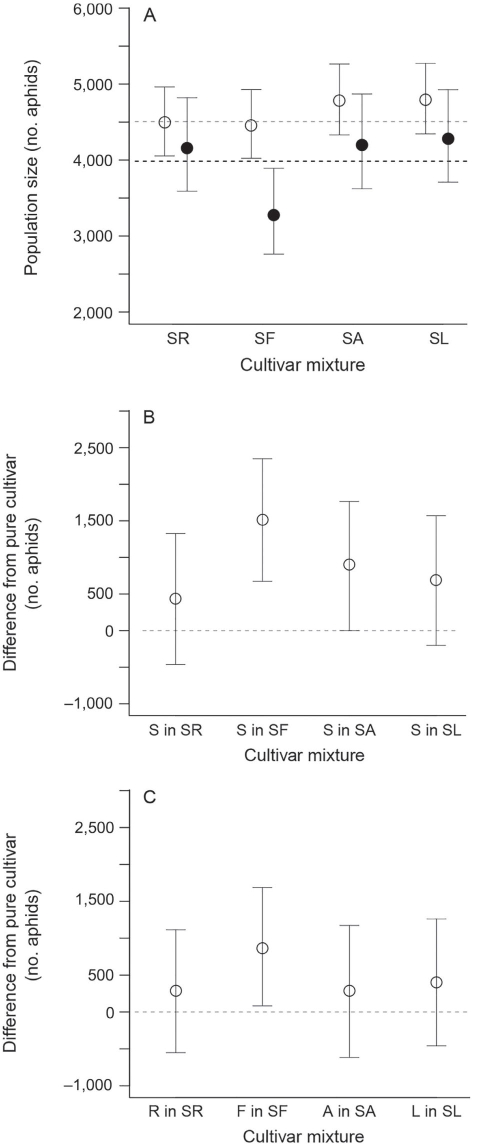

FIG. 1. (A) Expected and observed aphid population size per plot in mixtures and (B and C) differences between individual barley cultivars grown in monocultures and mixtures, estimated by the population model (mean with 95% CI). (A) Expected (empty symbols) and observed (black symbols) aphid population sizes in cultivar mixtures with the average estimated population size in monocultures (gray line) and mixtures (black line); (B) Salome (S) grown with other cultivars; (C) the other cultivars (A, F, R) grown with Salome (S). Positive differences indicate higher population sizes in monocultures.

sensitive to slight changes in their host plants, and plant neighbor responses can affect their behavior and abundance (Ninkovic et al. 2016).

Volatile organic compounds

Based on the reported variable effects of plant diversity on herbivores (Letourneau et al. 2011), and previous studies of VOC-mediated plant–insect interaction (Ninkovic et al. 2013), we hypothesized that plant–plant interaction via VOCs could be a powerful driver of variation in herbivore responses to cultivar mixtures. We compared the VOC profiles of studied cultivars and found that they differed significantly, which might explain the cultivar-combination-specific effects observed in many studies. Mutual interactions between Salome and Fairytale influenced aphid settling and population growth, but this cultivar pair could not be singled out based on the observed differences in overall odor profiles (Fig. 2). Among individual components, significantly higher amounts of (Z)-3-hexenyl acetate, linalool, linalool oxide, (–)-sativene, β-caryophyllene, (3E, 7E) - 4, 8, 12-trimethyl-1, 3, 7, 11-tridecatetraene (TMTT), methyl salicylate, and 1-octen-3-ol were observed in Salome, but only TMTT and hexahydrofarnesyl acetone were also relatively abundant in Fairytale. Hexahydrofarnesyl acetone has been previously identified as a plant volatile (Miyazawa et al. 2008), but no role in plant volatile signaling has been reported; the ecological significance of this compound may warrant further study. Further investigation of how specific VOCs induce plant growth responses and alter cultivar odor profiles will enable us to clarify the mechanisms behind plant–plant and plant–insect interactions.

Aphid population development

Population development of *R. padi* is related to cereal crop phenology. During colonization (between plant seedling and tillering), the population increases slowly and interference during this window has the greatest impact on aphid population size (Wiktelius and Pettersson 1985, Wiktelius et al. 1990). Aphids evaluate host plant quality after quick probes and leave plants if they find them to be less suitable (Schwarzkopf et al. 2013). A possible mechanism behind the observed patterns could be changes in aphid behavior caused by response to multiple sensory cues, such as complex volatile blends, i.e., the neural constraints hypothesis (Bernays 2001). This might mean in our case either that winged colonizers land less frequently or lay fewer nymphs in some mixtures, or that wingless aphids spend less time feeding relative to moving. Considering that there was no difference in colonization patterns (Appendix S4: Table S1) or in initial aphid abundance (Fig. 3), the former seems an unlikely explanation. Reduced feeding time in certain mixtures, however, might negatively influence population growth and contribute to the observed patterns. Aphid–plant acceptance is affected by visual, olfactory, and gustatory cues, but also by plant quality as a food source, which may change due to growth responses (Douglas and Van Emden 2007). Volatiles of another species/genotype are known to induce morphological and physiological responses in plants (Ninkovic 2003, Ninkovic et al. 2016), and since we observed changed aphid response to cultivars in certain mixtures, this suggests that the olfactory information emitted by plants was altered in these mixtures.

A population dynamics model for *R. padi* developed by Wiktelius and Pettersson (1985) showed that a 20% reduction

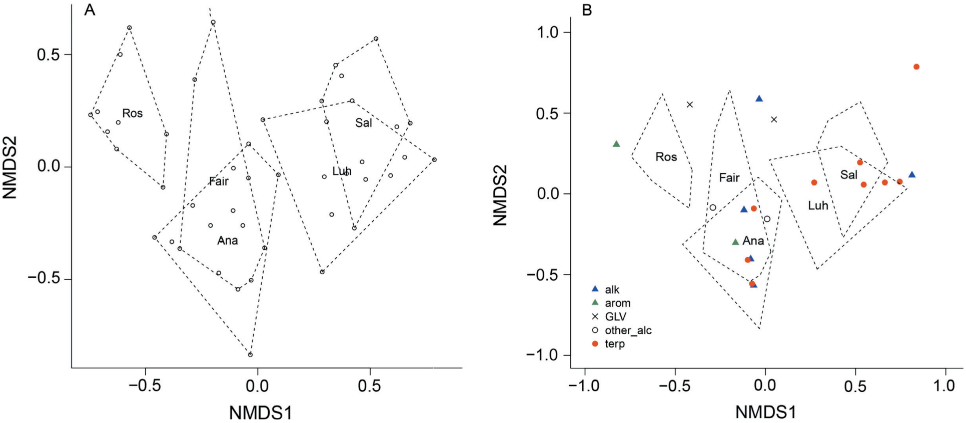

Fig. 2. Nonmetric multidimensional scaling (NMDS) results illustrating compositional dissimilarity in odor profiles between barley cultivars Anakin (Ana), Salome (Sal), Fairytale (Fair), Rosalina (Ros), and Luhkas (Luh). (A) Individual plants are shown with black circles and group boundaries for cultivar types marked with dashed lines using the ordihull function in vegan (Oksanen et al. 2017). (B) Individual components, categorized into alk, alkanes; arom, aromatic compounds; GLV, green leaf volatiles; other_alc, other alcohols; terp, terpenoids, with cultivar group boundaries same as in A.

in aphid numbers during plant acceptance can lower the final population size by 29%. This is in line with our current results, where the combination Salome–Fairytale resulted in reduced aphid acceptance in the laboratory (Table 1) and in 27% lower aphid populations in the field compared with monocultures of these cultivars (Fig. 1). This confirms the correlation between plant acceptance and subsequent population growth (Ninkovic et al. 2009, Dahlin and Ninkovic 2013), indicating that aphid development is suboptimal on plants on which they are reluctant to settle. We also observed aphid responses in the Salome–Anakin mixture, but the direction of response differed between lab and field experiments; aphid–plant acceptance was lower on Anakin whereas population development was reduced on Salome. This suggests that this interaction is weaker and more sensitive to differences between lab and field conditions; plants in the field can interact mutually and are exposed to competition, whereas only one-way interaction occurs in the lab.

Aphid population responses in the field conformed to all of our hypothesized outcomes: (1) most mixtures had only weak effects on aphids, (2) Salome responded to Anakin with limited (13%) reduction of aphid population size in the mixture, (3) Salome and Fairytale interacted with each other with a strong effect (31% and 22%, respectively) on aphids in the mixture. Slower aphid population growth on the responding cultivars was observed early in the season, and this was related to final population size (Fig. 1). The results from our experiments demonstrate that cultivar mixing per se gives no guarantee of decreased aphid populations, which concurs with a study by Grettenberger and Tooker (2017), who found that effects of genotypic diversity on aphid populations were inconsistent between experiments. However, we have shown that the effect on aphids depends on the ability of the cultivars in the mixture to respond to each other. We found that, for a certain cultivar combination, pest response is highly consistent between lab and field experiments (Table 1 and Fig. 1).

Aphid populations in mixtures were lower than expected from the average of monocultures, suggesting that a positive mixture effect is caused by interactions between cultivars; however, this effect was mainly driven by induced changes in aphid response to Salome. A significant reduction, which could be of practical relevance to agriculture, was only observed in the Salome–Fairytale mixture, where aphid–plant acceptance and population growth was reduced on both mixture components. Properties of different components in mixtures are important for disease and pathogen management, which relies on mixing plants differing in resistance (Mundt 2002). A positive mixture effect is achieved mainly through the dilution effect, as the resistant component reduces pathogen spread on the susceptible component. This mechanism cannot explain aphid-reduction patterns in our study. Even though aphid populations varied between cultivar monocultures (S, A, and L were higher than R and F; Table 2), patterns of population reduction were not correlated with this apparent variation intolerance (e.g., combination of cultivars with low tolerance [SA] had the same population size as a combination of cultivars with high and low tolerance [SR]). Thus, our results suggest that the identity of cultivars is of decisive importance for pest suppression in mixtures, but the driving mechanism is not based on variation in resistance between cultivars.

Aphid natural enemies

Pest population development may be regulated by top-down processes. Several studies on genotype mixtures (Johnson et al. 2006, Cook-Patton et al. 2011, Grettenberger and Tooker 2017) have supported the enemy hypothesis (Elton 1958), which predicts higher predator abundance in diverse stands. We found no relationship between predator abundance, aphid abundance, and cultivar combination. This indicates that the effects on aphid populations in our system

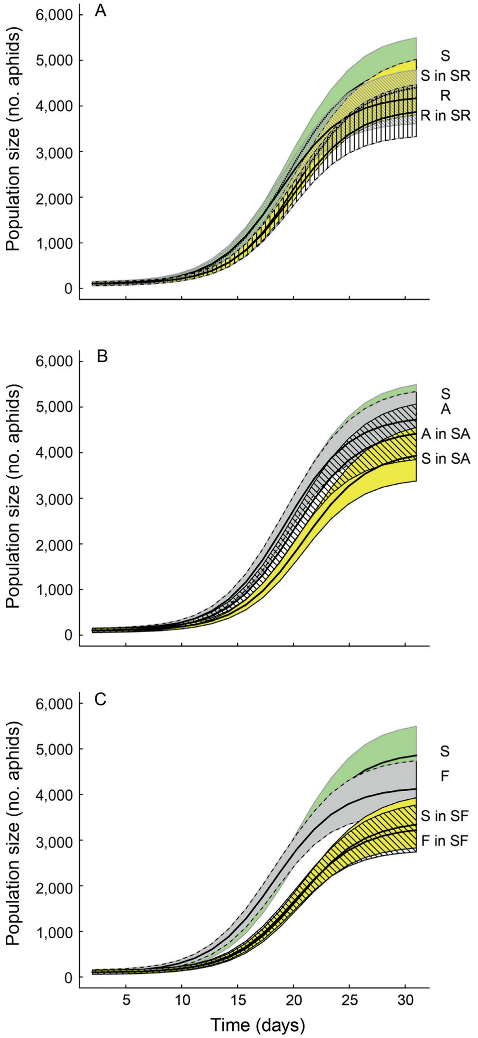

FIG. 3. Aphid population development overtime, estimated by the population model (mean with 95% CI) for (A) Salome (S) and Rosalina (R), (B) Salome (S) and Anakin (A), and (C) Salome (S) and Fairytale (F). Green indicates Salome in monocultures and yellow indicates Salome in mixtures.

were plant-mediated (bottom-up) and not natural-enemy-mediated (top down). We addressed the lowest level of diversity, i.e., mixtures of two cultivars, and the effect of enemies may be different in more diverse systems with alternative food sources or improved habitats. However, studies reporting higher enemy diversity in genotypically diverse mixtures have not provided evidence that enemy abundance patterns affect herbivore abundance (Crutsinger et al. 2006, Johnson et al. 2006, Cook-Patton et al. 2011), thus the potential of natural enemies to reduce herbivore populations in genotype mixtures is unclear.

CONCLUSIONS

Our findings provide new understanding of variation in herbivore responses to genotypically diverse fields and its basis in plant–insect interactions. We report interactive effects of genotype mixtures on pest population development, and show that the magnitude of the mixture effect is cultivar combination-specific, and consistent with changes in aphid–plant acceptance. The observed effect of VOC-induced changes on aphid–plant acceptance and differences in plant VOC profiles indicate that plant interactions lead to changed host plant physiology/aphid performance, which affects population development and determines the level of pest suppression. Thus, we propose that plant-mediated bottom-up effects drive plant–pest interactions in genotype mixtures, and that selecting cultivars based on how they interact with each other (both cultivars function as emitter and receiver) can be a promising sustainable pest management strategy. Future research should focus on testing cultivar responses to specific VOCs that potentially mediate interactions between plants, and quantifying plant growth responses, to build a predictive framework for identifying cultivars that, when mixed, reduce pest damage in agricultural crops.

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Supporting Information

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1807/full

Data Availability

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.224h5kr

Appendices

Appendix S1: Breeding information

Table S1. Breeding information about the tested two-row spring barley cultivars.

Cultivar	Breeder	Pedigree
Anakin	Sejet Plant Breeding, DK	Tumbler x Respons
Fairytale	Sejet Plant Breeding, DK	Colston x (Recept x Power)
Luhkas	R.A.G.T. Seeds Ltd, UK	Annabell x Prestige
Rosalina	Sejet Plant Breeding, DK	Beatrix x Eskobar
Salome	Nordsaat Saatzucht GmbH, DE	Auriga x (Publican x Beatrix)

Appendix S2: Collection and analysis of volatiles

Barley plants for the collection of VOCs were grown in plastic pots (10 × 10 × 7 cm) in potting soil (Special Hasselfors Garden (pH = 6.0), Hasselfors, Sweden) with 30 seeds per pot to produce sufficient biomass to generate measurable amounts of VOCs. Plastic pots were covered with aluminum foil and enclosed in a 60 × 55 cm PET oven bag (Toppits®, Melitta Scandinavian AB, Sweden). As a control, pots of soil were entrained. Teflon tubes were inserted through a hole in the bottom of the bags, sealed with rubber bands and air was pumped in through charcoal filters. A glass tube (80 × 3 mm) containing 0.05 g Porapak Q 60/80 mesh (Supelco Inc., Bellafonte, P.A.) was inserted in the top of the bag and connected to a pump by Teflon tubes. Air was pushed in/pulled out at a rate of 600/400 ml min^{-1} creating a positive pressure inside the bag and by that preventing entering contaminated air. Prior to entrainment, PET bags were baked in an oven at 140ºC for 2 h. Porapak tubes were heated at 140ºC and charcoal filters baked at 180ºC both under a flow of nitrogen to remove contaminants. Volatiles were collected in a climate chamber at 21ºC during 72 h. The treatments were replicated eight to ten times and the pots of soil two times.

VOCs were eluted from Porapak tubes with 750 µL redistilled dichloromethane and the sample was concentrated to 50 µL under nitrogen flow and stored at -20ºC until chemical analysis. Compounds were identified and quantified using coupled gas chromatography/mass spectrometry (GC/MS). A 3µl aliquot of each sample was injected onto a HP-1 column (30 m, 0.25 mm i.d., and 0.25 µm film thickness; J&W Scientific, Santa Clara, CA, USA) housed in a 7890A gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to an Agilent 5975C mass spectrometer. Ionization was by electron impact at 70 eV. The oven temperature was held at 30 °C for 1 min, then programmed at 5 °C min^{-1} to 150 °C, then at 10 °C min^{-1} to 250 °C. The carrier gas was helium with a flow rate of 1 mL min^{-1}. Identifications were made by comparison of spectra with a commercial database (NIST 2008) and by comparing mass spectra and retention times with those of authentic standards. Quantifications were made using response curves constructed with authentic standards.

Chemical standards were obtained as follows: (Z)-3-hexen-1-ol (98 %), (Z)-3-hexenyl acetate (98 %), linalool oxide (mixture of isomers) (97 %), 1-octen-3-ol (98 %), 6-methyl-5-hepten-2-one (99 %), naphthalene (99 %), tridecane (>99 %), undecane (>99 %), tetradecane (>99 %), pentadecane (>99 %), linalool (97%), methyl salicylate (98%), (-)-sativene (99%), 3-methyl-1-butanol (>99%) (all from Sigma-Aldrich, Sweden), β-myrcene (90%), (E)-β-caryophyllene (98.5%) (both Fluka, Sweden), hexahydrofarnesyl acetone (98%, Bedoukian, Danbury, CT, USA). A standard for the tentatively identified 3,5,5-trimethyl-2-

hexene was not available and this compound was quantified using 4-methyl-1-hexene (>98% Sigma-Aldrich). Standards of (E)-ocimene and (E,E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT) were kindly provided by Dr Mike Birkett, Rothamsted Research, UK.

Appendix S3: Model output testing aphid-plant acceptance

Table S1. Fixed effect estimates of aphid plant acceptance from generalized mixed models. Emitting cultivars marked by * were tested in a separate trial due to space limitation in the laboratory; a separate control treatment was present during each trial; n=40 replicates with treatment plants and n=20 replicates with control plants were used for each receiving cultivar. Significant effects (p<0.05) are marked in bold.

Receiving cultivar	Emitting cultivar	Estimate	SE	z	p
A	F	-0.73	0.25	-2.9	**0.003**
	L*	-0.2	0.24	-0.8	0.4
	R	-0.65	0.25	-2.6	**0.008**
	S	-0.91	0.24	-3.8	**0.0001**
F	A*	0.1	0.23	0.4	0.6
	L	-0.24	0.28	-0.9	0.4
	R	-0.4	0.27	-1.5	0.1
	S	-0.69	0.27	-2.6	**0.009**
L	A	-0.4	0.28	-1.4	0.2
	F*	0.14	0.23	0.6	0.5
	R	-0.3	0.28	-1.1	0.3
	S	-0.7	0.27	-2.6	**0.007**
R	A*	-0.05	0.23	-0.2	0.8
	F	0.02	0.24	0.1	0.9
	L	0.03	0.24	0.1	0.9
	S	-0.1	0.23	-0.5	0.6
S	A	-0.47	0.31	-1.5	0.1
	F	-0.95	0.3	-3.1	**0.002**
	L*	0.46	0.23	1.9	**0.05**
	R	-0.31	0.32	-1	0.3

Appendix S4: Model output testing aphid immigration

Table S1. Generalised linear mixed model output testing differences of aphid immigration to different cultivars and mixtures in the field; n = 45; * - cultivar Salome was used as reference category and estimate values for Salome obtained by setting SA as reference (because it had the highest estimate value) and rerunning the model; random effect and intercept estimates are the same for both models.

Fixed effects	Estimate	St Error	z	p
intercept	1.76	0.36	4.83	$1.3 \times e^{-6}$
SR	-0.13	0.52	-0.25	0.8
SF	-0.11	0.52	-0.21	0.8
SA	0.01	0.51	0.02	0.98
SL	-0.47	0.52	-0.9	0.37

Fixed effects	Estimate	St Error	z	p
R	-0.7	0.53	-1.3	0.2
F	-0.08	0.52	-0.17	0.87
A	-0.01	0.51	-0.03	0.97
L	-0.08	0.51	-0.16	0.87
S*	-0.01	0.51	-0.15	0.99
Random effects	**Variance**	**Std. Dev.**		
block	1.1 × e^{-10}	1.1 × e^{-5}		
observation	0.86	0.92		

Appendix S5: Model output testing predatory abundance effects on aphid numbers

Table S1. Generalised linear mixed model output testing effect of predatory arthropod abundance on aphid abundance in the field; n = 45.

Fixed effects	Estimate	St Error	z	p
intercept	8.25	0.11	71.9	2 × e^{-16}
Predator abundance (scaled)	0.02	0.06	0.29	0.78
Random effects	**Variance**	**Std. Dev.**		
block	0.05	0.22		
observation	0.13	0.36		

Appendix S6: Model output testing differences in predator numbers in pure cultivars and cultivar mixtures, and effects of aphid abundance on predator numbers

Table S1. Generalised linear mixed model output testing differences in predatory arthropod abundance in pure cultivars and mixtures in the field, and effect of aphid abundance on predator abundance; n = 45. Cultivar Salome was initially used as reference category, followed by setting SA as reference (because it had the highest estimate value) and rerunning the model; estimates from SA reference model are shown, because it contained the only significant difference between cultivars; * - estimate for SA was obtained from Salome reference model; random effect and intercept estimates are the same for both models. AIC was lower for random factor and intercept-only model (ΔAIC >5).

Fixed effects	Estimate	St Error	z	p
intercept	4.32	0.17	25.6	2 × e^{-16}
Aphid abundance (scaled)	0.09	0.1	0.85	0.4
SR	-0.03	0.12	-0.24	0.8
SF	-0.18	0.13	-1.38	0.17
SA*	0.17	0.13	1.3	0.2
SL	-0.2	0.12	-1.6	0.11
R	-0.2	0.13	-1.93	0.053
F	-0.19	0.12	-1.56	0.12
A	-0.16	0.13	-1.23	0.22
L	-0.4	0.13	-3.08	0.002
S	-0.17	0.13	-1.3	0.2
Random effects	**Variance**	**Std. Dev.**		
block	0.03	0.17		
observation	0.02	0.13		

Appendix S7: Mean values and standard deviation of predator abundance

Table S1. Mean number and standard deviation of predatory arthropod abundance in the different treatment plots.

Treatment	**Mean values**	**Standard deviation**
Salome	7.0	0.83
Rosalina	6.9	0.84
Fairytale	6.7	0.80
Anakin	7.1	0.81
Luhkas	6.5	0.79
Salome with Rosalina	7.4	0.82
Salome with Fairytale	6.6	0.79
Salome with Anakin	7.3	0.83
Salome with Luhkas	7.3	0.86

Appendix S8: Volatile compounds of the different cultivars

Table S1. Identified and quantified compounds (mean ng ± SE) in the headspace of the different cultivars. Significant differences ($p < 0.05$) are indicated with letters a - c.

Compound	**Salome**	**Anakin**	**Fairytale**	**Luhkas**	**Rosalina**
(Z)-3-hexen-1-ol	0.1 ± 0.04^{ab}	0.03 ± 0.01^{b}	0.07 ± 0.05^{ab}	0.04 ± 0.02^{b}	0.2 ± 0.05^{a}
(Z)-3-hexenyl acetate	0.3 ± 0.1^{a}	0.02 ± 0.004^{c}	0.04 ± 0.02^{c}	0.09 ± 0.03^{b}	0.08 ± 0.02^{b}
β-myrcene	0.1 ± 0.02^{a}	0.04 ± 0.004^{c}	0.07 ± 0.01^{b}	0.1 ± 0.02^{a}	0.07 ± 0.01^{b}
Linalool	0.9 ± 0.1^{a}	0.06 ± 0.01^{b}	0.09 ± 0.03^{b}	0.6 ± 0.2^{a}	0.05 ± 0.009^{b}
Linalool oxide	0.1 ± 0.01^{a}	0.007 ± 0.002^{c}	0.02 ± 0.004^{c}	0.03 ± 0.006^{b}	0.01 ± 0.003^{c}
(z)-ocimene	0.01 ± 0.001^{a}	_	_	0.01 ± 0.002^{a}	_
(E)-ocimene	0.05 ± 0.01^{a}	0.009 ± 0.008^{b}	_	0.1 ± 0.02^{a}	_
(-)-sativene	0.02 ± 0.007^{a}	0.0009 ± 0.0009^{b}	_	_	_
β-caryophyllene	0.2 ± 0.1^{a}	_	_	0.004 ± 0.004^{b}	_
TMTT*	0.007 ± 0.002^{a}	_	0.003 ± 0.0007^{b}	_	0.0007 ± 0.0003^{c}
Hexahydrofarnesyl-acetone	0.1 ± 0.03^{a}	0.05 ± 0.006^{b}	0.1 ± 0.01^{a}	0.06 ± 0.02^{b}	0.1 ± 0.02^{a}
Undecane	0.07 ± 0.03^{a}	0.03 ± 0.005^{ab}	0.05 ± 0.01^{ab}	0.05 ± 0.02^{ab}	0.02 ± 0.008^{b}
Tridecane	0.04 ± 0.02^{a}	0.02 ± 0.004^{a}	0.03 ± 0.008^{a}	0.03 ± 0.02^{a}	0.02 ± 0.006^{a}
Tetradecane	0.04 ± 0.01^{a}	0.04 ± 0.01^{a}	0.06 ± 0.02^{a}	0.09 ± 0.05^{a}	0.03 ± 0.02^{a}
Pentadecane	0.09 ± 0.03^{a}	0.09 ± 0.03^{a}	0.1 ± 0.05^{a}	0.2 ± 0.1^{a}	0.07 ± 0.03^{a}
3,5,5-trimethyl-2-hexene	0.003 ± 0.003^{ab}	_	0.01 ± 0.005^{a}	0.002 ± 0.002^{ab}	0.003 ± 0.003^{ab}
Naphthalene	0.03 ± 0.006^{a}	0.02 ± 0.003^{ab}	0.03 ± 0.005^{ab}	0.03 ± 0.009^{ab}	0.01 ± 0.004^{b}
Methyl salicylate	0.2 ± 0.02^{a}	0.06 ± 0.01^{b}	0.1 ± 0.02^{b}	0.09 ± 0.02^{b}	0.09 ± 0.02^{b}
1-octen-3-ol	0.4 ± 0.07^{a}	0.008 ± 0.004^{bc}	0.03 ± 0.01^{b}	0.02 ± 0.009^{b}	0.008 ± 0.008^{c}
3-methyl-1-butanol	0.04 ± 0.005^{b}	0.02 ± 0.003^{b}	0.02 ± 0.002^{c}	0.03 ± 0.004^{b}	0.4 ± 0.03^{a}