

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

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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).

Manuscript received 18 April 2018; revised 14 August 2018; accepted 22 August 2018. Corresponding Editor: Matthew P. Ayres.

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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/m² 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 watertraps (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:

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

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

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

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

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

$$h_p \sim \text{Normal}(\mu_{h_p}, \sigma_{h_p})$$

where μ is the mean number of aphids per plot, V_{max} is the maximum (final) aphid population size, h is half-time (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 \pm SE) to 4846 ± 327

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.

Receiver	Emitter				
	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 (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

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).

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 half-time (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 (Δ AIC = 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.

Treatment	Aphid population size (V_{\max})			Confidence	Halftime, h (d)		
	Mean	2.5% CI	97.5% CI		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

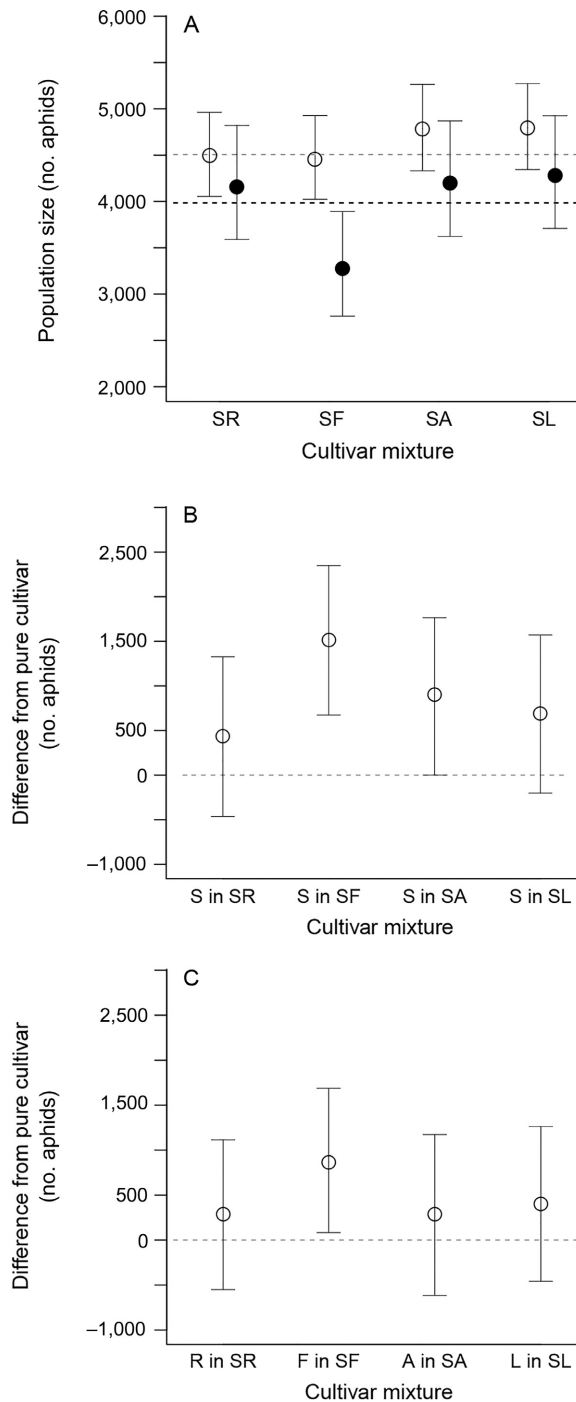


FIG. 1. (A) Expected and observed aphid population size per plot in mixtures and (B and C) differences between individual barley cultivars grown in monocultures and mixtures, estimated by the population model (mean with 95% CI). (A) Expected (empty symbols) and observed (black symbols) aphid population 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, (3*E*, 7*E*)-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

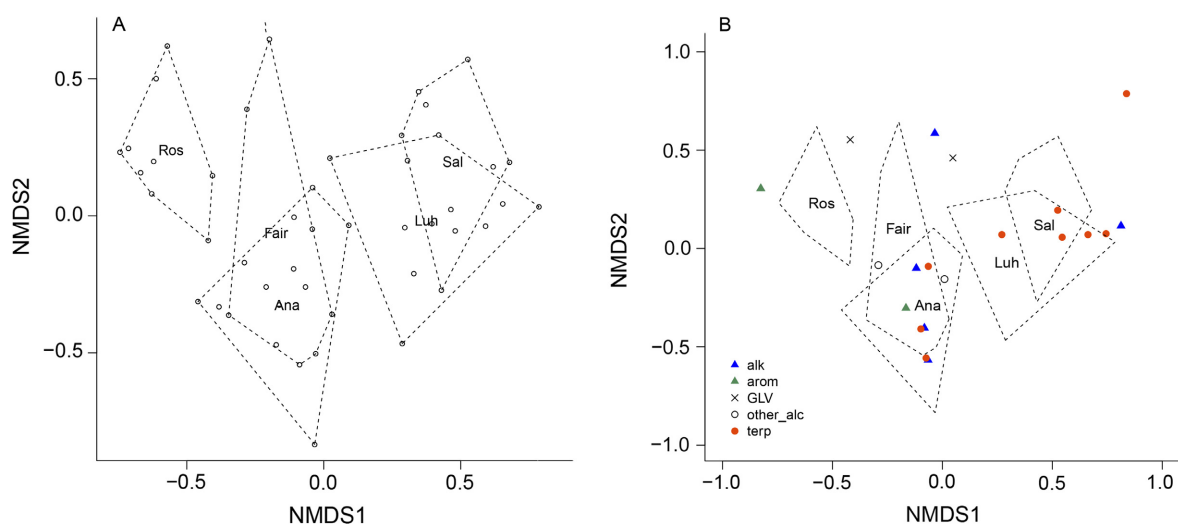


FIG. 2. Nonmetric multidimensional scaling (NMDS) results illustrating compositional dissimilarity in odor profiles between barley cultivars Anakin (Ana), Salome (Sal), Fairytale (Fair), Rosalina (Ros), and Luhkas (Luh). (A) Individual plants are shown with black circles and group boundaries for cultivar 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

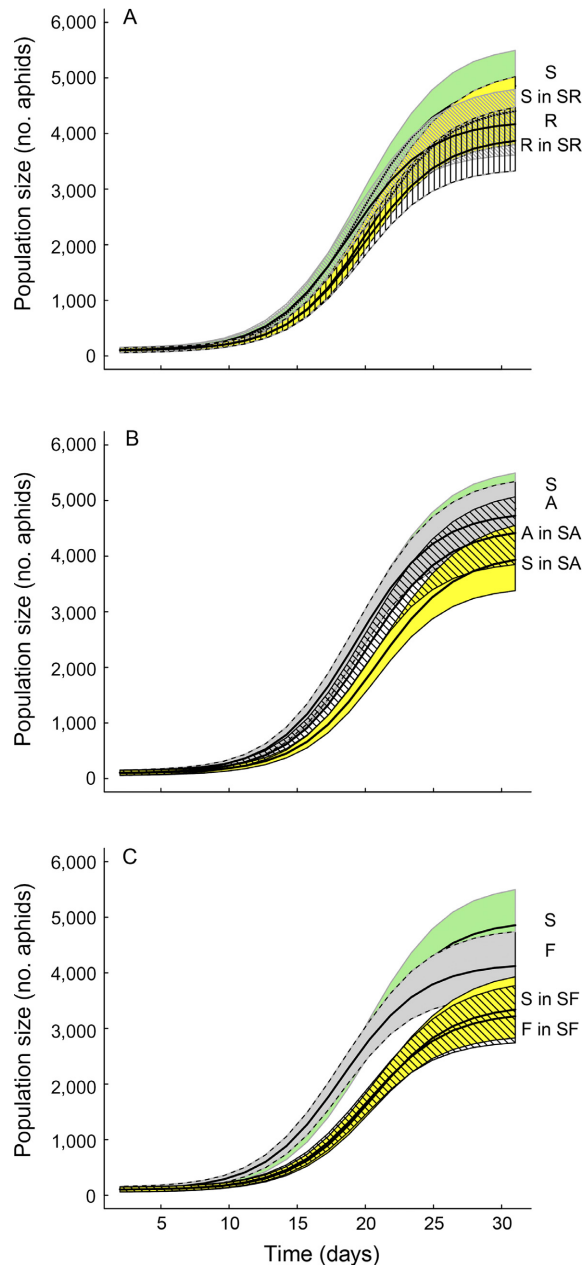


FIG. 3. Aphid population development overtime, estimated by the population model (mean with 95% CI) for (A) Salome (S) and Rosalina (R), (B) Salome (S) and Anakin (A), and (C) Salome (S) and Fairytale (F). Green indicates Salome in monocultures and yellow indicates Salome in mixtures.

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.

ACKNOWLEDGMENTS

The study was financially supported by the Swedish Research Council for Sustainable Development, FORMAS (2014-225) and the Foundation Land Use Research (SLF) (H1333072). We thank James Ajal, Bara'a Alkhaled, Ali El Hakeem, H  l  ne Lamaison, Ewa Magnuski, and Dimitrije Markovic for field assistance and Maria Kedmark for taxonomic surveys. We greatly acknowledge advice from   sa Ranlund, Jonas Knape, and Matt Low on population modelling. Special thanks to Anna M  rtensson, G  ran Nordlander, and Niklas Bj  rklund for their comments on the manuscript. Iris Dahlin and Diana Rubene contributed equally to this work as authors. Authors declare no conflict of interest.

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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>