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Synergies and trade-offs between insect and pathogen resistance in maize leaves and roots

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ABSTRACT

Determining links between plant defence strategies is important to understand plant evolution and to optimize crop breeding strategies. Although several examples of synergies and trade-offs between defence traits are known for plants that are under attack by multiple organisms, few studies have attempted to measure correlations of defensive strategies using specific single attackers. Such links are hard to detect in natural populations because they are inherently confounded by the evolutionary history of different ecotypes. We therefore used a range of 20 maize inbred lines with considerable differences in resistance traits to determine if correlations exist between leaf and root resistance against pathogens and insects. Aboveground resistance against insects was positively correlated with the plant's capacity to produce volatiles in response to insect attack. Resistance to herbivores and resistance to a pathogen, on the other hand, were negatively correlated. Our results also give first insights into the intraspecific variability of root volatiles release in maize and its positive correlation with leaf volatile production. We show that the breeding history of the different genotypes (dent versus flint) has influenced several defensive parameters. Taken together, our study demonstrates the importance of genetically determined synergies and trade-offs for plant resistance against insects and pathogens.

Key-words: *Zea mays*; herbivore-induced plant volatiles; induced defence; pathogens; resistance; root herbivory; synergy.

INTRODUCTION

In nature, plants are constantly challenged by a multitude of organisms. Insect herbivores feeding on leaves and roots, for instance, represent a significant threat, as do the many pathogenic microorganisms. It is therefore not surprising that plants have evolved a diverse array of defence and tolerance strategies to fend off and cope with the different attacks (Rausher 2001). The synthesis of toxins (Koul 2008) and volatile organic compounds (VOCs) (Kant *et al*. 2009), as well as the formation of structural barriers like lignified cell walls, trichomes and callose deposits, is among the most common defences found in plants (Karban & Baldwin 1997). Tolerance mechanisms include, for example, the diversion of assimilates to non-attacked tissues (Schwachtje & Baldwin 2008) and an increased capacity for regrowth after attack (Núñez-Farfán, Fornoni & Valverde 2007).

One central question that arises from this defensive diversity is whether the different defence and tolerance strategies are independent of each other or whether they show positive or negative associations. Understanding genetic dependence is of considerable ecological interest because it can determine the type of selection pressure that attackers impose on a host plant (Rausher 1996; Strauss, Sahli & Conner 2005) as well as the structure of plant-associated communities (Leimu & Koricheva 2006). Plant breeding for resistance may also benefit from unravelling genetic links between defence traits (Mitchell-Olds *et al*. 1995), as the same quantitative trait loci (QTLs) or breeding markers may be exploited for different resistance features. Several scenarios of genetic dependence are possible, and examples have been reported for all of them. Firstly, if defences are specifically tailored against one herbivore and are costly to the plant, there may be trade-offs between the different strategies. *Phaseolus lunatus*, for example, shows a negative association between cyanogenesis, a direct defence, and volatile release (Ballhorn *et al*. 2008). Secondly, if the defences are non-specific, the same mechanism may increase the plant's capacity to withstand different attackers, leading to synergistic or positive associations. For instance, iridoid glycosides in *Plantago lanceolata* are effective against both insects and biotrophic fungal pathogens (Biere, Marak & van Damme 2004). Similarly, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a hydroxamic acid produced by gramineous plants, deters insects and reduces pathogen growth *in vitro* (Rostas 2007). Thirdly, if the defences are specific, but neither involve a considerable cost nor exert any pleiotropy, they may act in parallel, leading to neutral effects. In *Populus tremuloides*, concentrations in condensed tannins and the production of extrafloral nectar were found to be independent from each other (Wooley *et al*. 2007).

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Overall, the three possible linkages described above can come into effect in different environmental situations, and it is important to distinguish between at least two types of genetic dependencies: The first type involves a situation where a plant is under threat by multiple attackers, either sequentially or simultaneously (Bruce & Pickett, 2007). Depending on the available resources, the plant may have to prioritize its defensive investment. Furthermore, defensive pathways may constrain each other if they are interdependent. An example is the negative crosstalk between jasmonic acid (JA)- and salicylic acid (SA)-dependent pathways (Spoel, Johnson & Dong 2007). Other hormones involved in stress responses also interact, some antagonistically, others synergistically (Gazzarrini & McCourt 2003). From a meta-analysis of genetic correlations between plant resistance to multiple attack, it was concluded that the majority of the interactions are positive (Leimu & Koricheva 2006), although there was large variation in both strength and direction of the associations.

The second type of genetic dependency involves the capacity of a particular genotype to withstand attacks by single threats. Given the fact that insect and pathogen pressure are spatially and temporally variable, a plant may have to mount a specific defence response against one predominant threat, while its clone, sibling or offspring may have to cope with a different main attacker. Crosstalk and defence priorities are of no importance here, as only one attacker is present on a given plant, but genetic links may still have important consequences for its capacity to respond. Certain constitutive defences fall into this category: They are normally not targeted at a specific attacker and may either facilitate or constrain other plant resistance traits. Within the cotton clade (Gossypieae), for example, a negative correlation was found between the number of toxic gossypol glands and trichome density (Rudgers, Strauss & Wendel 2004). For induced responses, molecular dependencies may constrain or facilitate defensive strategies as well. A strongly responsive upstream signalling cascade, for example, may strengthen all the downstream defences, independently of their fine tuning. Ca^{2+}-mediated changes in plasma membrane potentials, for instance, are important for the onset of defence signalling against both pathogens and insects (Maffei, Mithofer & Boland 2007; Ma *et al*. 2008). Plants with a strong genetically fixed potential for such events can thus be expected to be very reactive to a broad range of stimuli, resulting in positive genetic links among induced resistance traits to single attackers. Similarly, specific variants of metabolic enzymes may produce secondary metabolites that either favour one or the other strategy. In maize, the channelling of indole is such an example: the indole-3-glycerol phosphate lyase (IGL) produces free indole that is released as a volatile upon attack (Frey *et al*. 2004), whereas the closely related enzyme BX1 (a tryptophane synthase homolog) channels indole towards the production of hydroxamic acids (Frey *et al*. 1997), which have a role in direct defences. Depending on the transcriptional activity of igl and bx1 and the activity, affinity and presence of the corresponding enzymes, a plant may therefore have a predetermined genetic capacity to invest either in volatile release or direct defence. Overall, as selective pressure can be imposed by both multiple attacks and different types of single attacks, both types of molecular links should be considered when trying to disentangle trade-offs and synergies between defensive strategies.

Compared to situations with multi-species attacks, genetically fixed correlations between defensive strategies against single attackers are hard to detect, as they are inherently confounded by the evolutionary history of different ecotypes or species (Rasmann & Agrawal 2009). For example, plants growing in tropical regions are likely to experience high risk of insect attack, as well as pathogen colonization. It is therefore very likely that positive associations are found between insect and pathogen resistance when comparing ecotypes or species from tropical and other climatic zones, not because there is a genetic link between resistance mechanisms but because traits have been selected for in parallel. Even correcting for the evolutionary history by using phylogenetic independent contrasts cannot fully resolve this problem. Although often regarded as less suitable from an ecological perspective (Voelckel & Baldwin 2004; Leimu & Koricheva 2006), crop plants may actually have some advantages here because their genetic make-up has been heavily reshuffled as a result of artificial selection and breeding; it can be expected that many parallel traits have been eliminated, especially as the main selection criteria over the last century has been yield and product quality rather than insect and pathogen resistance (Donald & Hamblin 1976). In crop plants like maize, where hybrids are used for crop production, an impressive genetic diversity among inbred lines and landraces has been maintained (Wang *et al*. 1999). Furthermore, crop breeding has provided a diversity of non-segregating independent lines, which makes it possible to carry out well-standardized experiments to evaluate differences between genotypes. With natural ecotypes and collected accessions that are still segregating, this is much more difficult. Crop plants such as maize, with all their inherent limitations linked to artificial selection, may in fact represent useful models to study genetic dependence of resistance and tolerance.

Taking advantage of the unique mix of dissociation and diversity in maize, we profiled an extensive set of resistance and tolerance parameters in 20 maize inbred lines and a hybrid in order to get insight into genetic linkage, synergies and trade-offs between different defence patterns. By measuring direct and indirect defences as well as tolerance against pathogens and herbivores in the leaves and roots in fully independent experiments, we aimed at profiling and correlating a large set of defence strategies against single attackers. The data set enabled us to tackle the following specific questions: (1) Are there links between insect and pathogen resistance?; (2) Are there trade-offs between direct defences and volatile release?; (3) Are leaf and root defences correlated?; and (4) Does the breeding history (flint versus dent) explain differences in resistance traits? This study does not only provide answers to the preceding questions, but also describes a number of unique resistance factors in some of the inbred lines that warrant further investigation.

MATERIALS AND METHODS

Plants, insects and pathogens

Twenty different inbred lines of maize (*Zea mays* L.) from Delley semences et plantes SA (DSP, Switzerland) were used for the experiments. Half of the lines were flint maize (*Z. mays* indurate) and the other half was classified as dent lines (*Z. mays* indentata). For comparative purposes, we also included the flint hybrid 'Delprim' into the assays. For convenience, the different lines were named alphabetically from A to V. More information about their genetic background is available from Delley DSP upon request. The inbred lines and the hybrid were sown in plastic pots (11 cm height, 4 cm diameter) with commercial soil (Aussaaterde, Ricoter, Aarberg, Switzerland; for exceptions see below) and grown in a climate chamber at 25 ± 2 °C, 60 ± 5% r.h., 16:8 h l:d, and 400 μmol m^{-2} s^{-1}. Maize plants used for the experiments were 10–14 d old and had three fully developed leaves.

Two insect and two fungal pathogen species were used for the experiments. Eggs of the leaf-herbivore *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) were provided by Syngenta (Stein, Switzerland), and the larvae were reared on artificial diet as previously described (Turlings, Davison & Tamo 2004). The eggs and larvae of the root herbivore *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) were obtained from the United States Department of Agriculture Agricultural Research Service North Central Agricultural Research Laboratory (USDA-ARS-NCARL, Brookings, USA) and kept on freshly germinated maize seedlings until use. The fungal pathogen *Colletotrichum graminicola* (Sordariomycetes: Glomerellaceae) was obtained from the University of Kentucky (USA). The wild-type strain of *C. graminicola* (Ces) C.W. Wils (M1.001) and its transgenic derivatives were maintained at 25 °C on potato dextrose agar (PDA) medium (Difco PDA, Becton, Dickinson and Company, Le Pont de Claix, France) under continuous illumination (70 μmol m^{-2} s^{-1}). For long-term storage, 4 mm agar plugs were suspended into 50% glycerol and kept at −80 °C. The necrotrophic fungus *Setosphaeria turcica* Leonard et Suggs (Pleosporales: Pleosporaceae) was cultivated on modified PDA medium (9.75 g PDA and 1.5 g agar for 500 mL). Sporulation was induced by culturing 1 week in a phytotron (21 ± 1 °C, 12:12 h l:d, 200 μmol m^{-2} s^{-1}) followed by 2 weeks in the dark at room temperature.

Green fluorescent protein transformation of *C. graminicola*

To facilitate the measurement of pathogen colonization, we expressed green fluorescent protein (GFP) in *C. graminicola*. Wild-type protoplasts were transformed with the plasmid gGFP (McCluskey 2003), which contains the green fluorescent plasmid (GFP) driven by the promoter of the *Aspergillus nidulans gpd* gene, and the *amp* and *hph* gene conferring resistance to ampicillin and hygromycin B. Protoplasts were prepared and transformed according to a published protocol (Sukno *et al*. 2008). Transformants were selected on PDA containing 150 μg/mL hygromycin B. After genetic purification by single-sporing transgenic candidates, the transformants were phenotyped for colony morphology, growth rate and virulence.

Leaf-herbivore resistance

To measure leaf-herbivore resistance, the different maize lines were grown as described above. Experiments were carried out under light benches in a climatized laboratory (25 ± 2 °C, 40 ± 10% r.h., 16:8 h l:d, and 148 μmol m^{-2} s^{-1}). Individual plants were infested with four neonate *S. littoralis* larvae, for which equal starting weight was assumed ($n = 12$). To stop the larvae from escaping, poly-ethylene (PET) bottles with the bottom cut out (30 cm height, cone-shaped, maximum diameter 8 cm) were placed upside down over the plants and attached to the pots with Parafilm. The tubes were open at the top to guarantee air circulation. After 5 d of feeding, the *S. littoralis* larvae were removed from the plants and weighed. The total larval weight per plant was recorded and divided by the number of larvae to yield average individual weight. After weighing, the *S. littoralis* caterpillars were put back on the same plants. After another 4 d of feeding, the herbivores were removed and the plants scored for survival. Plants were rated 'alive' when they still had green tissue or the stem was intact. Plants that had been completely destroyed, including the removal of the stem below the growing point, were considered dead. Since larvae on dead plants were not able to feed *ad libitum* any more, final larval weight after 9 d was not considered for analysis.

Root herbivore resistance

To facilitate the recovery of the root herbivores, plants were sown in sand (3–5 mm, JUMBO, Neuchâtel, Switzerland) topped with 2 cm of commercial soil (Aussaaterde, Ricoter, Aarberg, Switzerland) in this assay. Previous studies have shown no effect of the soil type on the reaction of maize plants to *D. virgifera* attack (Erb *et al*. 2009a). The seedlings were fertilized once at 2 d after germination using MioPlant Vegetable and Herbal Fertilizer (MIGROS, Zurich, Switzerland) to compensate for the reduced nutrient density compared to soil-grown plants. When the seedlings were 12 d old, five second-instar *D. virgifera* larvae were weighed and placed on the soil ($n = 9$). The pots were covered with aluminium foil at the top and the bottom to prevent the larvae from escaping. After 7 d, the leaves of plants were scored for wilting symptoms using a scale from 0 (no wilting) to 4 (complete loss of turgidity). The soil and roots were removed from the pots and *D. virgifera* larvae were recovered. The larvae were placed on a dry filter paper to remove excess moisture from their cuticle and weighed. Total weight gain was determined and the average individual weight gain was calculated.

Resistance to *C. graminicola*

To evaluate the resistance of the different maize lines against *C. graminicola*, the second true leaf of each 12-day-old plant was cut and transferred to Petri dishes filled with 2% agar solution. Each leaf was inoculated with six separate drops of a *C. graminicola* gGFP spore solution [10^7 conidia/mL, 0.01 M $MgSO_4$, 0.01% Silwet L-77 (Lehle Seeds, Round Rock, TX, USA)]. After inoculation, the Petri dishes were transferred to a climate chamber (25 ± 2 °C, 16:8 h l:d, 200 μmol m^{-2} s^{-1}). Five days post-inoculation, eight leaves from each line were randomly chosen and the infection spots were observed with a dissecting microscope (Nikon model C-BD230, Nikon Instruments, Inc., Tokyo, Japan). GFP fluorescence was excited with blue light (430–470 nm). Images were captured using the Nikon digital sight DS-L1 device. Pictures were further processed using Photoshop (Adobe Systems Inc.) to measure GFP areas.

Resistance to *S. turcica*

To assess plant resistance against *S. turcica*, 12-day-old plants were challenged: the second true leaf of each plant was cut and placed on agar plates as described above and was inoculated with 5 μL droplets of 0.01 M $MgSO_4$ 0.01% Silwet-L77 containing 5×10^4 spores/mL, by applying three drops on each detached leaf. Eight plants per variety were infected. After inoculation, the detached leaves were placed in darkness for 16 h at high RH (90%) and room temperature. Then, all plates were transferred to a phytotron (25 ± 2 °C, 60 ± 5% r.h., 16:8 h l:d, 400 μmol m^{-2} s^{-1}). Four days after inoculation, infection spots were observed with a microscope (Nikon model SMZ1000) under normal light, and images were captured as above. Disease severity was quantified by measuring lesion length using ImageJ (http://rsbweb.nih.gov/ij/). In an additional experiment, the resistance of the lines against *S. turcica* was measured over 5 d of infection. Second and third true leaves of 14-day-old plants were cut and placed on 2% water agar plates. Detached leaves were inoculated with two 10 μL droplets of 0.01 M $MgSO_4$ 0.01% Silwet-L77 containing 5×10^4 spores/mL. After inoculation, detached leaves were kept in darkness for 16 h at high RH (90%) and room temperature. Then, all plates were transferred to a phytotron (21 ± 2 °C, 60 ± 5% r.h., 12:12 h l:d, 200 μmol m^{-2} s^{-1}). Five days after inoculation, disease severity per plant was quantified for four plants per variety by measuring lesion lengths of both challenged leaves with ImageJ.

Root volatiles

Plants for root volatile measurements were grown under similar conditions as for the root resistance assays (see earlier discussion). To measure volatile production without the confounding effect of differential insect activity on the different lines, we chose to induce the plants artificially. As no root herbivore-derived elicitors are known and roots cannot be artificially damaged without heavily impairing their physiology, induction was accomplished by drenching the soil with 10 mL of a JA solution to a final concentration of 250 μM ($n = 6$). JA accumulates in the roots after *D. virgifera* attack (Erb *et al*. 2009a) and is commonly used to mimic herbivore attack. Control plants were drenched with 10 mL of water. After 24 h, the roots were collected, washed with water and frozen in liquid nitrogen. The roots were ground with a bead mill using glass beads, and 0.3 g of the obtained powder was placed into a glass vial with a septum-containing lid. Samples were stored at –80 °C. For volatile analysis, a 100 μm polydimethylsiloxane solid-phase microextraction (Supelco, Bellefonte, PA, USA) fibre was inserted through the septum and exposed to the vial headspace for 20 min at 35 °C. The compounds adsorbed on the fibre were then analysed by gas chromatography-mass spectrometry (GC–MS) with an Agilent 6890 Series GC system G1530A (Palo Alto, CA, USA) coupled to a quadrupole mass-selective detector (Agilent 5973; transfer line 230 °C, source 230 °C, ionization potential 70 eV). The fibre was inserted into the injector port (250 °C) and desorbed and chromatographed on an apolar column (DB1-MS, 30 m, 0.25 mm internal diameter, 0.25 μm film thickness; J & W Scientific, Folsom, CA, USA). Helium at a constant pressure of 50.6 kPa was used as carrier gas. After fibre insertion, the column temperature was maintained at 60 °C for 1 min and then increased to 220 °C at 10 °C min^{-1} followed by a final stage of 3 min at 250 °C. The volatiles were identified by comparing their mass spectra with those of the NIST05 Mass Spectra Library and by comparing retention times and MS spectra with those of pure compounds and previous analyses.

Leaf volatiles

To measure leaf volatiles without the confounding effect of differential resistance, we artificially induced the leaves. To mimic herbivore attack, the abaxial side of all leaves (20 mm^2) of six plants per maize line was scratched with a scalpel blade without damaging the midrib. Subsequently, 10 μL of *S. littoralis* larval regurgitant was applied to each wound using a micropipette. Regurgitant had previously been collected with a micropipette from fourth-instar *S. littoralis* larvae that had been feeding on maize leaves for at least 24 h and the regurgitant was stored at –80 °C until use. Control plants were left undamaged ($n = 3$ per maize line). Plants were put in glass bottles and connected to a multiple air delivery system and odours were trapped on a Super-Q trap (25 mg, 80–100 mesh; Alltech Associates, Deerfield, IL, USA) as described elsewhere (Turlings *et al*. 2004). Traps were washed with 3 mL dichloromethane before each collection period. Purified air entered the bottles at a rate of 1.1 L min^{-1} and air carrying the volatiles was pulled through each trap at a rate of 0.7 L min^{-1}. Volatiles were collected during two sampling periods per day, the first one from 7:00 to 13:00 and the second one from 15:00 to 21:00. After each sampling period, the traps were extracted with 150 μL dichloromethane (Super solvent; Merck, Dietikon, Switzerland), and 200 ng of *n*-octane and *n*-nonyl acetate (Sigma, Buchs, Switzerland) in 10 μL dichloromethane were added to each sample as internal standards.

Aliquots of 2 μL of each sample were injected into a gas chromatograph (Agilent 7890A) coupled to a mass spectrometer (Agilent 5975C VL MSD with Triple-Axis Detector; transfer line 230 °C, source 230 °C, ionization potential 70 eV) in pulsed splitless mode onto a non-polar column (HP-1 MS, 30 m, 0.25 mm ID, 0.25 μm film thickness, Alltech Associates, Inc). Helium at constant flow (0.9 mL min^{-1}) was used as carrier gas. After injection, the temperature was maintained at 40 °C for 3.5 min, then increased to 100 °C at 8 °C min^{-1} and subsequently to 200 °C at 5 °C min^{-1} followed by a post-run of 5 min at 250 °C. The volatiles were identified by comparing their mass spectra with those of the *NIST05* library and by comparing their retention times with those of previous analyses. The total emission for each compound was calculated by summing up the calculated concentrations for both collection periods (0–6 h and 8–14 h after induction). In addition, to control for the different leaf biomass of the lines, emissions were divided by the average weight of the different genotypes after 10 d to yield emission g^{-1} fresh weight.

Statistical procedures

Prior to every statistical analysis, a Levene's and a Kolmorogov–Smirnov test were carried out to determine heteroscedasticity of error variance, normality and the appropriate data transformation. When data sets did not pass the tests, square root or $\log_{10}$ transformation was carried out (for data transformation, see individual tests in the results section). Kruskal–Wallis or Mann–Whitney rank sum tests were used for data sets that did not fulfil the assumption for analyses of variance (ANOVAs) after transformation. The Holm–Sidak procedure was used for post hoc testing following ANOVAs. Proportions of surviving plants were compared with a chi-square test. Flint and dent lines were compared using mean values per genotype. All tests mentioned above were carried out using SigmaPlot 11.0. Additionally, for root volatiles, a principal component (PC) analysis was adjusted following the procedure described previously (Held, Gase & Baldwin 2004). To determine the appropriate model for description of gene distribution, a detrended correspondence analysis was performed. The given dimensionless value for the length of gradient of the first ordination axis was <3, indicating that the values should be fitted by a linear distribution model. Therefore, PC analysis (PCA) was based on a linear model. PCA was performed using the Canoco 4.5 package (Ter Braak & Smilauer 2002). Correlations were carried out for mean values per genotype using Pearson product moment correlations (quantitative comparisons) and Spearman rank order correlations (qualitative comparisons).

RESULTS

Phenotypic differences

Overall, the growth conditions were suitable for all the tested maize lines, as they developed normally without any stress symptoms. Germination was above 65% for most lines, with the exception of variety R, of which less than 50% of the plants germinated. Growth varied between the different lines: Average fresh weight after 10 d was between 1.5 g (variety J) and 2.7 g (variety N). The hybrid Delprim clearly grew faster than the inbred lines, reaching an average fresh weight of 3.5 g.

Resistance and tolerance to leaf herbivory

Larval weight after 5 d of feeding was used to estimate plant resistance against the generalist leaf-feeder *S. littoralis*. At this point, sufficient leaf biomass was still available for consumption in all lines. Plant survival after 9 d was taken as a cumulative measure of herbivore resistance and tolerance. Overall, there was a significant effect of plant genotype on larval growth (Fig. 1a; $F_{20} = 3.926$; $P < 0.001$), with a twofold difference between the lowest average weight (variety V, 7.7 mg) and the highest average weight of the larvae (variety E, 13.9 mg). Similarly, plant survival was considerably different between genotypes (Fig. 1b, d.f. = 20; $\chi^2 = 44.862$; $P < 0.001$), with 100% survival of variety I and 11% of variety K. Herbivore growth and survival of the maize seedlings was negatively correlated (Spearman $\text{cor.coeff}_{21} = 0.468$; $P = 0.0318$; $R^2 = 0.217$): Fewer individual plants were still alive 9 d after herbivore attack in genotypes that supported faster herbivore growth compared to more resistant lines.

Resistance and tolerance to root herbivory

Root resistance and tolerance was measured after 7 d of *D. virgifera* infestation by determining larval weight gain and evaluating wilting symptoms. At this point, many plants had suffered heavily from root attack, and clear wilting symptoms could be observed aboveground. However, living root tissue was still available to *D. virgifera* in all lines, indicating that biomass limitation was not responsible for differences in weight gain. Overall, there was no significant genotype effect on larval growth (Fig. 1c; Kruskal–Wallis test, $H_{20} = 21.102$, $P = 0.391$), even though some lines showed a trend to increased *D. virgifera* resistance (Fig. 1c). The plant genotypes differed significantly in the extent of leaf wilting after root attack (Fig. 1d; Kruskal–Wallis test, $H_{20} = 65.550$; $P < 0.001$): While lines E and H, for example, remained green and turgid, several varieties were strongly wilting and clearly suffered heavily from *D. virgifera* attack. Overall, flint isolines wilted significantly more than dent lines after *D. virgifera* attack (*t*-test, $t_{18} = -2.121$, $P = 0.048$).

Resistance to pathogens

The total surface of *C. graminicola* infested tissue varied significantly between genotypes (Fig. 2b; Kruskal–Wallis

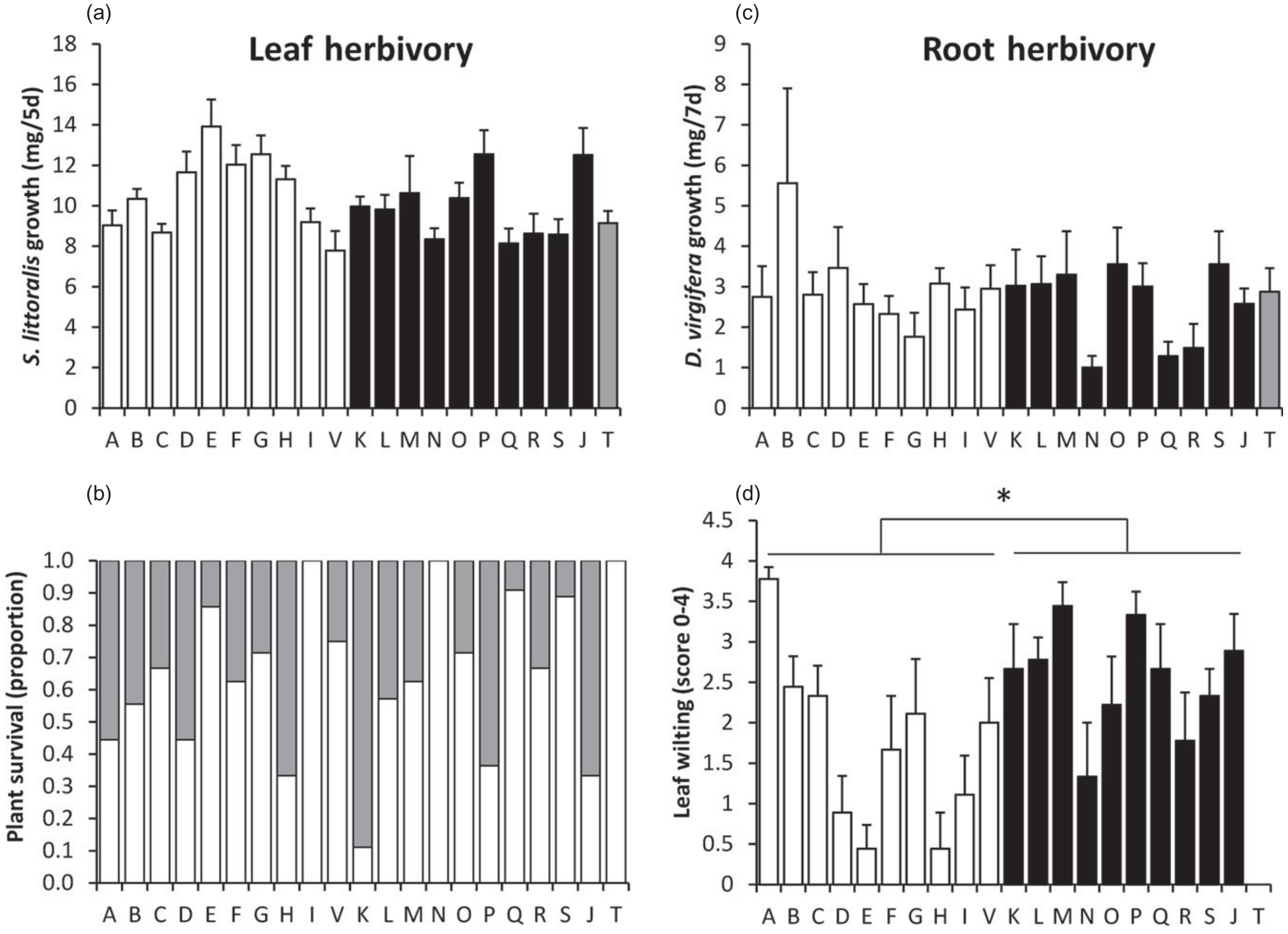

Figure 1. Influence of plant genotype on herbivore resistance and tolerance. White bars: dent lines; black bars: flint lines; grey bars: Delprim (hybrid). (a) Average growth (+SE) of the leaf-herbivore *Spodoptera littoralis* on the different lines. (b) Proportion of surviving plants after 9 d of leaf-attack (white bars) opposed to dying plants (grey bars). (c) Average growth (+SE) of the root herbivore *Diabrotica virgifera* on the different lines. (d): Average leaf-wilting score after root herbivore attack (Scale from 0 = no wilting to 4 = heavy wilting). Asterisk denotes significant differences between genotype groups (dent versus flint; $P < 0.05$).

test, $H_{20} = 103.587$, $P < 0.001$). Overall, flint genotypes were almost twice as susceptible as dent lines (*t*-test, $t_{18} = -2.457$, $P = 0.024$). Lesion sizes caused by *S. turcica* after 4 d of infection was also significantly different between the tested lines ($\log_{10}$ transformed, $F_{20} = 15.113$, $P < 0.001$), with line A being four times more susceptible than the average genotype (Fig. 2a, Holm–Sidak post hoc test A versus any other genotypes: $P < 0.001$). No significant correlation between plant resistance against *S. turcica* and *C. graminicola* could be detected (Pearson cor.coeff$_{21}$ = 0.375, $P = 0.093$; Spearman cor.coeff$_{21}$ = 0.400; $P = 0.071$). Interestingly, genotypes that had smaller *S. turcica* lesions showed a higher growth rate of *S. littoralis* (Spearman cor.coeff$_{21}$ = –0.452, $P = 0.0393$). To explore this phenomenon in more detail, we also measured lesion sizes of *S. turcica* infested lines after 5 d using a higher concentration of spores. Resistance against *S. turcica* after 4 and 5 d was strongly correlated (Pearson corr.coeff$_{21}$ = 0.661, $P = 0.001$, $R^2 = 0.444$; Fig. 2c). Again, a significant negative correlation could be found between *S. littoralis* growth and *S. turcica* lesion size after 5 d of infestation (Pearson cor.coeff$_{21}$ = –0.500, $P = 0.0210$, $R^2 = 0.250$; Spearman corr.coeff$_{21}$ = –0.506, $P = 0.019$, $R^2 = 0.256$; Fig. 2d).

Root volatiles

After drenching 10-day-old maize seedlings with a JA solution, we found 12 compounds in the roots that were not present in water-treated controls. On the basis of earlier work and available standards, seven VOCs were identified (Table 1). The structure of five other metabolites was only tentatively assigned by comparison of mass spectra with the *NIST05* library. Four representative compounds are shown in Fig. 3: the two major root sesquiterpenes (*E*)-*β*-caryophyllene and (*E*)-*α*-copaene, the major aldehyde hexadecanal as well as geosmin, a yet undescribed root volatile. Interestingly, flint lines produced significantly more (*E*)-*α*-copaene (Mann–Whitney rank sum test, U = 16.5, $P = 0.010$), (*E*)-*β*-caryophyllene (Mann–Whitney rank sum test, $U = 17.0$, $P = 0.014$), *α*-humulene (Mann–Whitney rank

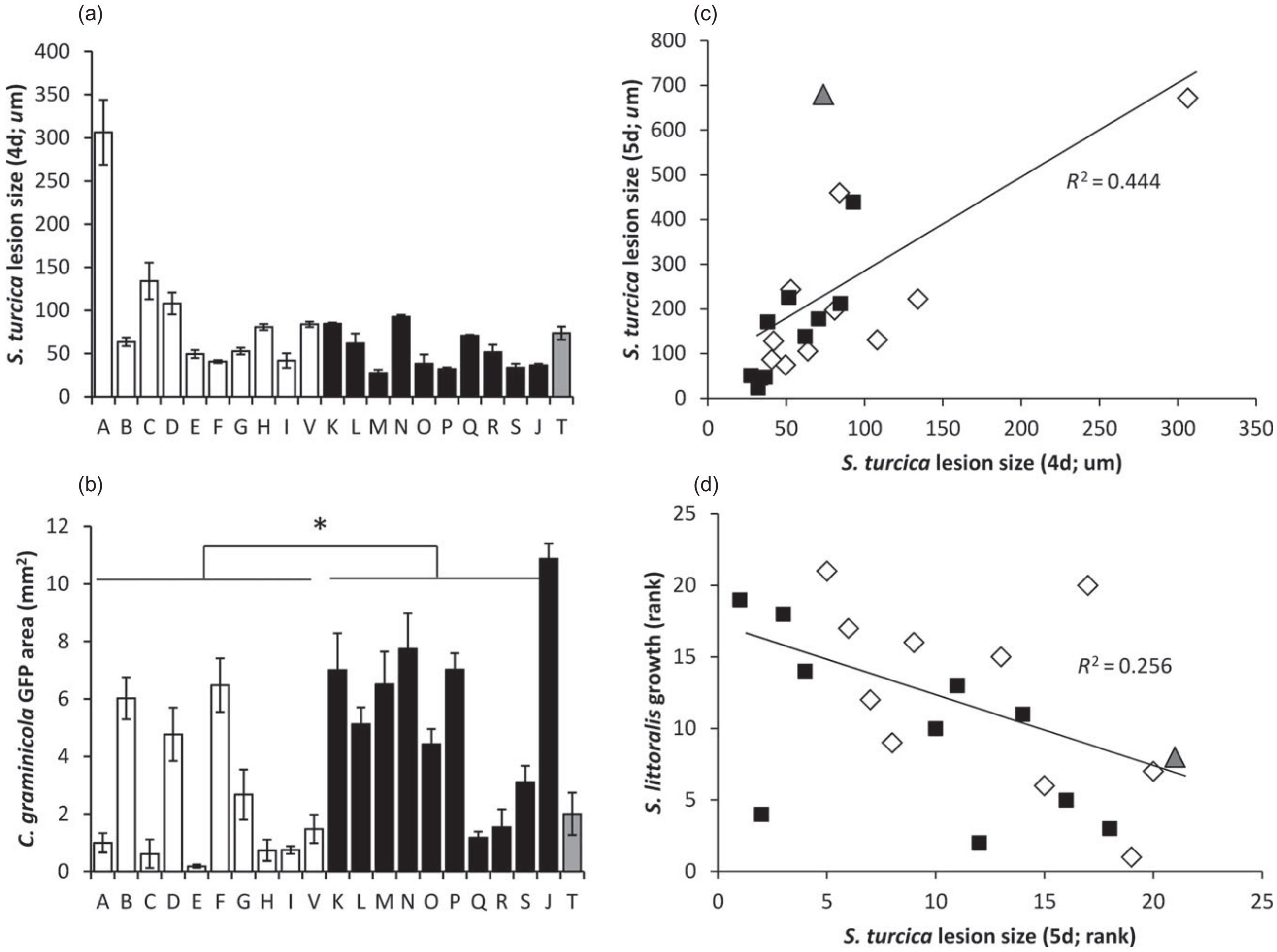

Figure 2. Influence of plant genotype on pathogen resistance. White bars: dent lines; black bars: flint lines; grey bars: Delprim (hybrid). (a) Average lesion size (±SE) caused by *Setosphaeria turcica* on the different lines. (b) Average GFP area (±SE) caused by *Colletotrichum graminicola* on the different lines. (c) Correlation between average lesion sizes per genotype caused by *S. turcica* after 4 and 5 d of infection (two separate experiments). (d) Correlation between *S. littoralis* growth and *S. turcica* lesion size. Genotypes are ranked from 1 (smallest lesions, weakest herbivore growth) to 21 (biggest lesions, strongest herbivore growth). Linear regression lines and R^2 goodness-of-fit values are shown. Asterisk denotes significant differences between genotype groups (dent versus flint; $P < 0.05$).

sum test, $U = 17.0$, $P = 0.014$), (*E*)-α-cubebene (Mann–Whitney rank sum test, $U = 12.0$, $P = 0.002$) and another unknown sesquiterpene than dent lines (Mann–Whitney rank sum test, $U = 18.0$, $P = 0.013$). For the JA-induced plants, strong correlations were found between the quantity of different compounds (means per variety, Pearson product moment correlations, $P < 0.05$, Table 2). The highest correlation coefficients were measured for the two products of the TPS23 enzyme (Köllner *et al.* 2008), (*E*)-β-caryophyllene and α-humulene (Table 2, Fig. 4a), followed by a group consisting of the two TPS8 products (*E*)-α-copaene and (*E*)-α-cubebene and the unknown sesquiterpenes (Table 2, Fig. 4b). The emission of alkanes and aldehyde compounds (hexadecanal, tetradecanal, tetradecane and an unknown aldehyde) was positively correlated within the group but negatively correlated with the sesquiterpenes (Table 2; Fig. 4c). Indole and geosmin were produced independently from the other VOCs. These patterns were also visible in a PCA, where the vector space was divided into the same four groups of VOCs (Fig. 4d). Axis 1 explained 87% of the variability, and axis 2 explained 12%. Dent lines clustered together as low producers of sesquiterpenes and high producers of aldehydes and alkanes, while flint lines were more variable, but tended to have a higher sesquiterpene production (Fig. 4d). Variety K was clearly separated from the rest (see 'K' in Fig. 4d), mostly because of its high production of hexadecanal and tetradecanal. Root volatile emission upon JA treatment was not correlated with resistance against *D. virgifera* for any of the 12 compounds (Pearson and Spearman corr.coeff$_{21}$: $P > 0.05$).

Leaf volatiles

To measure the emission of leaf volatiles, maize seedlings were induced by scratching and application of *S. littoralis* regurgitant. In total, 24 compounds were found to be induced in the different varieties (Table 1). Most of them have been identified and described before (Degen *et al.*

Table 1. Average quantities of induced leaf- and root volatiles

	Roots (peak area)		Leaves (ng/12 h)	
Compound	Constitutive	Induced	Constitutive	Induced
Indole	n.d.	2.84E + 05	n.d.	2.12E + 02
(*E*)-*α*-copaene	1.03E + 05	2.06E + 06	2.09E + 00	2.49E + 00
Geosmin	3.09E + 05	1.83E + 06	n.d.	n.d.
Tetradecane	1.60E + 05	6.05E + 05	n.d.	n.d.
(*E*)-*β*-caryophyllene	2.16E + 05	7.57E + 06	5.48E + 00[b]	2.15E + 02
α-humulene	5.62E + 03	3.71E + 05	n.d.	2.85E + 00
Sesquiterpene[a]	2.46E + 04	2.05E + 05	n.d.	n.d.
(*E*)-*α*-cubebene	1.81E + 05	6.38E + 05	n.d.	n.d.
Aldehyde[a]	5.99E + 05	2.18E + 06	n.d.	n.d.
Sesquiterpene II[a]	2.15E + 05	6.58E + 05	n.d.	n.d.
Hexadecanal[a]	1.68E + 06	7.79E + 06	n.d.	n.d.
Tetradecanal[a]	1.41E + 07	4.42E + 07	n.d.	n.d.
(*E*)-2-hexenal	n.d.	n.d.	n.d.	1.78E − 01
(*Z*)-3-hexen-1-ol	n.d.	n.d.	n.d.	7.93E − 01
β-myrcene	n.d.	n.d.	1.56E + 00[b]	4.36E + 00
(*Z*)-3-hexenyl acetate	n.d.	n.d.	4.49E + 00[b]	1.76E + 01
Linalool	n.d.	n.d.	4.08E + 01	2.14E + 02
DMTT	n.d.	n.d.	1.34E + 01[b]	3.46E + 02
2-Phenthenyl acetate	n.d.	n.d.	n.d.	7.30E + 01
Methyl antranilate	n.d.	n.d.	n.d.	2.64E + 00
Indole	n.d.	n.d.	n.d.	2.12E + 02
Gernanyl acetate	n.d.	n.d.	n.d.	1.22E + 01
Cycloisosativene[a]	n.d.	n.d.	2.21E + 01	4.29E + 01
α-ylangene[a]	n.d.	n.d.	1.52E + 01	2.04E + 01
(*E*)-*α*-bergamotene	n.d.	n.d.	9.43E − 02[b]	2.74E + 02
Sesquiterpene[a]	n.d.	n.d.	n.d.	1.83E + 00
(*E*)-*β*-farnesene	n.d.	n.d.	n.d.	9.27E + 02
Germacrene D	n.d.	n.d.	8.35E + 00[b]	1.79E + 01
Sesquiterpene II[a]	n.d.	n.d.	n.d.	2.20E + 00
(*E,E*)-*α*-farnesene	n.d.	n.d.	n.d.	1.15E + 01
β-sesquiphellandrene	n.d.	n.d.	9.54E − 01[b]	2.07E + 01
Nerolidol	n.d.	n.d.	n.d.	5.96E + 01
TMTT	n.d.	n.d.	n.d.	2.51E + 01

Average values across individual plants and genotypes are shown. Volatiles that were encountered only exceptionally in uninduced leaves are marked with an asterisk. Volatiles are depicted according to their retention time on a non polar column (HP1-MS), with the root volatiles shown before the leaf-induced compounds.

[a] Denotes tentatively identified compounds.

[b] Traces in few samples.

2004). Four representative compounds of the main VOC families (green leaf volatiles, aromatic compounds, terpenoids) are shown in Fig. 5. Four compounds were induced in both leaves and roots: Indole (*E*)-*α*-copaene, (*E*)-*β*-caryophyllene and *α*-humulene. Upon induction, flint lines produced significantly more indole (Mann–Whitney rank sum test, $U = 20.5$, $P = 0.028$) (*E*)-*β*-caryophyllene (Mann–Whitney rank sum test, $U = 7.5$, $P < 0.001$), germacrene D (Mann–Whitney rank sum test, $U = 15.0$, $P < 0.002$) and *α*-farnesene (Mann–Whitney rank sum test, $U = 30.0$, $P < 0.035$). In contrast to the roots, only positive correlations were detected between the different induced volatiles (Table 3). The emission of compounds derived from the same biochemical pathway was often positively correlated (Fig. 6, Pearson product moment correlations, $P < 0.05$, see Table 3 for correlation coefficients). The products of the sesquiterpene synthase TPS10, for example, (*E*)-*α*-bergamotene and (*E*)-*β*-farnesene, were emitted in similar ratios (Fig. 6a). Interestingly, numerous positive correlations were also observed between biochemically more distant VOCs. The emission of the green leaf volatile (GLV) (Z)-3-hexenyl-acetate, for instance, was positively correlated with the sesquiterpene (*E*)-*α*-bergamotene (Fig. 6b), the monoterpene linalool and the homoterpene 4,8-dimethylnona-1,3,7-triene (DMNT) (Table 3). Correction for leaf-biomass resulted in a comparable pattern of correlations between volatiles (data not shown). PCA did not reveal any patterns of separation between flint and dent lines or between the different classes of VOCs (data not shown). Of the VOCs that were detected in both the leaves and the roots, the emitted quantities did not show any apparent correlations (Pearson and Spearman corr.co-eff$_{21} < 0.4 > -0.4$, $P > 0.05$). Spearman rank correlations, however, revealed a significant qualitative correlation between leaf and root emission of (*E*)-*β*-caryophyllene (cor.coeff$_{21} = 0.789$, $P < 0.001$, $R^2 = 0.646$, Fig. 6c), an effect

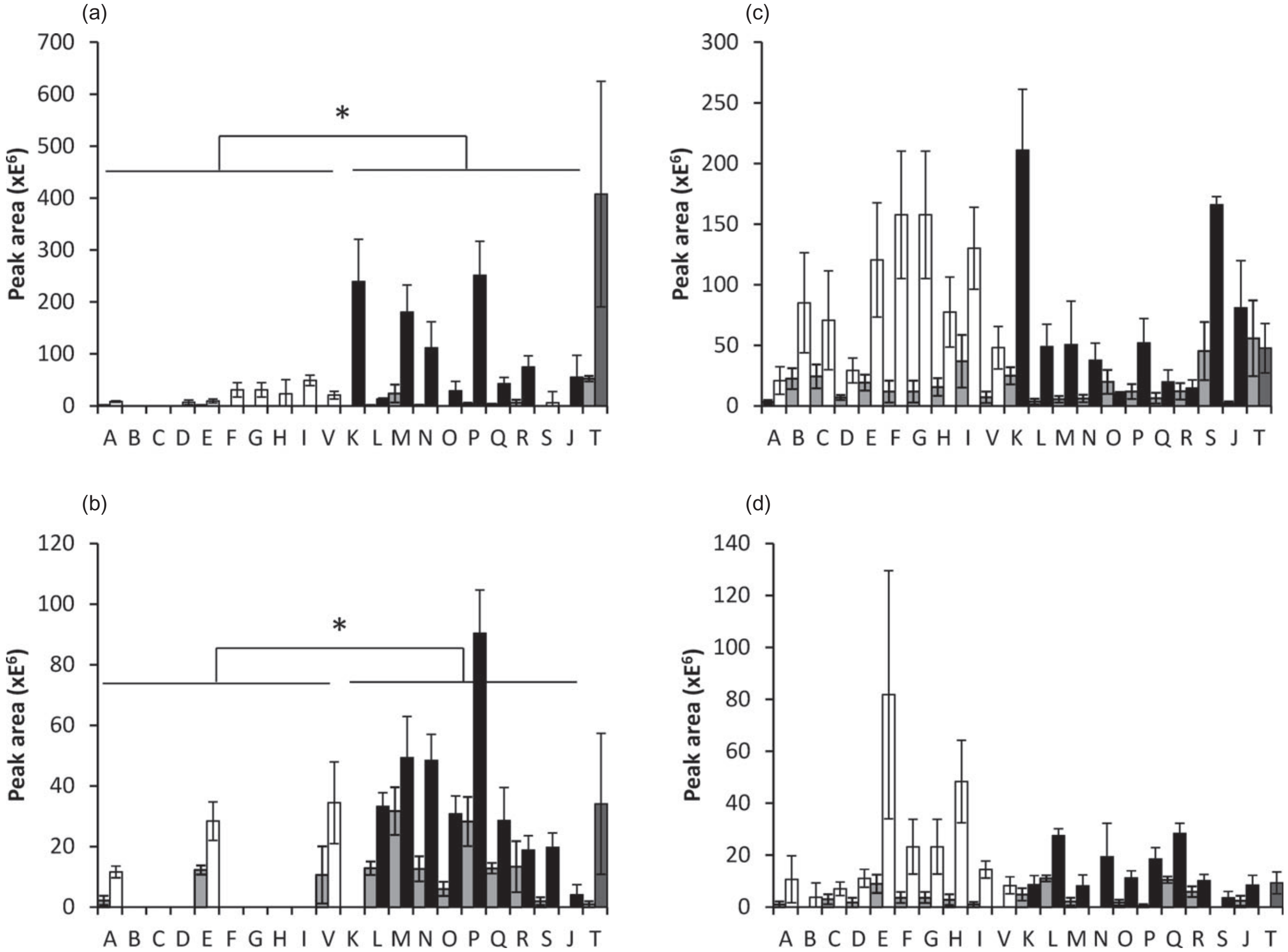

Figure 3. Emission of induced root volatiles. Average relative amounts (±SE) emitted by each genotype are shown for four representative volatile compounds. Emission of control plants (bright grey) and induced plants is shown. White bars: dent lines; black bars: flint lines; dark grey bars: Delprim (hybrid). (a) (*E*)-*β*-caryophyllene; (b) (*E*)-*α*-copaene; (c) hexadecanal; (d) geosmin. Asterisks denote significant differences between genotype groups (dent versus flint; $P < 0.05$).

Table 2. Correlation coefficients (Pearson product moment correlations) between 21 maize genotypes for the different induced root volatile compounds

	Compound	2	3	4	5	6	7	8	9	10	11	12
1	Indole	–0.13	0.34	–0.04	–0.05	–0.13	–0.11	–0.21	–0.09	–0.19	–0.09	–0.01
2	(*E*)-*α*-copaene		0.06	**–0.54**	**0.47**	**0.54**	**0.89**	**0.91**	–0.02	**0.97**	–0.42	–0.48
3	Geosmin			0.08	–0.20	–0.20	–0.04	–0.07	0.12	0.01	0.11	0.09
4	Tetradecane				–0.12	–0.22	–0.37	**–0.50**	**0.60**	**–0.56**	**0.81**	**0.73**
5	(*E*)-*β*-caryophyllene					**0.98**	**0.49**	**0.52**	0.09	0.40	0.01	0.09
6	*α*-humulene						**0.57**	**0.59**	0.06	**0.49**	–0.03	0.05
7	Sesquiterpene^N							**0.86**	0.05	**0.87**	–0.26	–0.25
8	(*E*)-*α*-cubebene								0.00	**0.94**	–0.38	–0.43
9	Aldehyde^N									–0.03	**0.84**	**0.69**
10	Sesquiterpene II^N										–0.42	**–0.50**
11	Hexadecanal^N											**0.91**
12	Tetradecanal^N	^{N}tentative identification										

Bold numbers indicate a significant correlation ($P < 0.05$).
N, tentative identification.

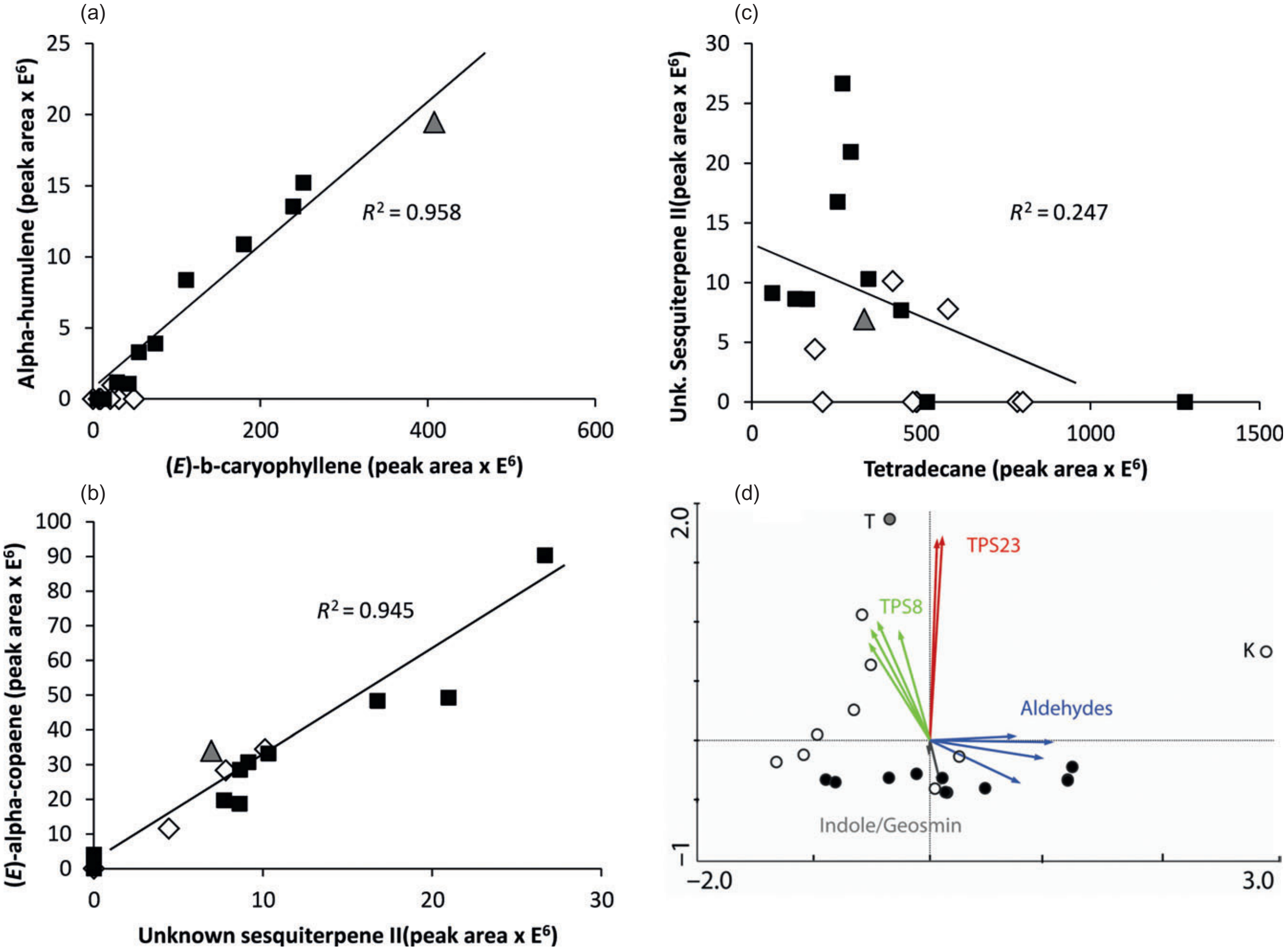

Figure 4. Correlations of root volatiles. White symbols: dent lines; black symbols: flint lines; grey symbols: Delprim (hybrid). (a) Correlation between the emission of (*E*)-*β*-caryophyllene and *α*-humulene in the different genotypes. (b) Correlation of (*E*)-*α*-copaene and an unknown sesquiterpene in the different genotypes. (c) Correlation of the emission of an unknown sesquiterpene and tetradecane in the different genotypes. Linear regression lines and R^2 goodness-of-fit values are shown. (d) Principal component analysis of average induced volatile emission of the different genotypes. Vectors of the different volatiles are separated according to grouping within the PCA and biosynthetic origin. Green: TPS8 products; red: TPS23 products; blue: aldehydes; grey: indole and geosmin 'K' denotes the outlier variety K.

that was equally strong when the leaf volatile data were corrected for biomass (data not shown). Interestingly, a clear trend was visible for a number of VOCs to be negatively correlated with *S. littoralis* growth (Pearson product moment correlations, indole: $\text{cor.coeff}_{21} = -0.400$, $P = 0.072$ (*E*)-*α*-bergamotene: $\text{cor.coeff}_{21} = -0.382$ $P = 0.0879$, *β*-sesquiphellandrene: $\text{cor.coeff}_{21} = -0.354$ $P = 0.0850$). The same trend was visible for the total sum of induced leaf volatiles (Pearson $\text{cor.coeff}_{21} = -0.391$ $P = 0.0796$). Further analysis showed significant Spearman rank correlations between *S. littoralis* growth and the emission of (*E*)-*α*-bergamotene ($\text{cor.coeff}_{21} = -0.432$ $P = 0.049$, $R^2 = 0.225$, Fig. 6d) and *β*-sesquiphellandrene ($\text{cor.coeff}_{21} = -0.550$ $P = 0.001$, $R^2 = 0.302$): *S. littoralis* larvae gained less weight on genotypes that had higher induced levels of these compounds. When corrected for leaf biomass, the same trend remained visible, albeit less strong for (*E*)-*α*-bergamotene ($\text{cor.coeff}_{21} = -0.400$ $P = 0.071$). As only eight of the 21 genotypes emitted detectable amounts of *β*-sesquiphellandrene, we also tested emitting against non-emitting plants for *S. littoralis* resistance. Overall, *β*-sesquiphellandrene-emitting plants supported less growth of *S. littoralis* than non-emitters (Mann–Whitney rank sum test, $U = 16.00$, $P = 0.001$).

DISCUSSION

Our experiments demonstrate that different resistance mechanisms in maize are genetically linked. Insect resistance in the leaves, measured via herbivore growth rates (Fig. 1a), was positively correlated with the plant's capacity to emit certain VOCs like (*E*)-*α*-bergamotene upon herbivory (Fig. 6d). To our knowledge, this is the first study to specifically report on a positive correlation between herbivore-induced plant volatiles (HIPVs) and insect resistance. As we did not find any direct negative effect of these

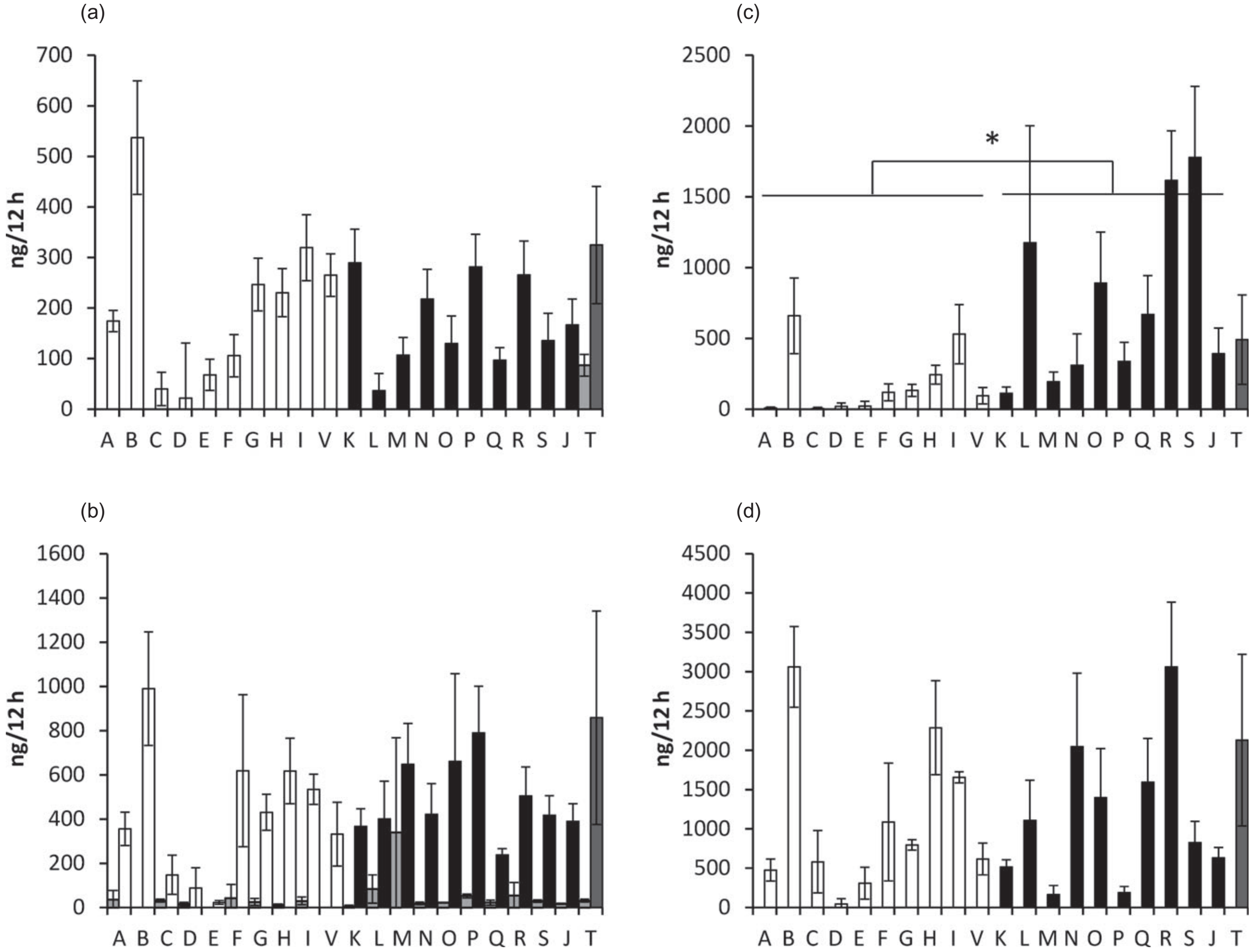

Figure 5. Emission of induced leaf volatiles. Average amounts (±SE) emitted by each genotype are shown for four representative volatile compounds. Emission of control plants (bright grey) and induced plants is shown. White bars: dent lines; black bars: flint lines; dark grey bars: Delprim (hybrid). (a) (*Z*)-3-Hexenyl acetate; (b) linalool; (c) indole; (d) (*E*)-*β*-farnesene. Asterisk denotes significant differences between genotype groups (dent versus flint; $P < 0.05$).

compounds on the growth of *S. littoralis* (G. von Merey and N. Veyrat, personal communication), we suggest that this correlation may be the result of a shared upstream signalling cascade between HIPVs and direct defences. It is known that the release of VOCs after herbivore attack is positively correlated with JA concentrations (Schmelz *et al*. 2003) and that JA application induces resistance against *S. littoralis* (Erb *et al*. 2009a). Our findings nicely match those of Shivaji *et al*. (2010), who compared two maize genotypes and showed that the more resistant line had higher induced JA levels. Hence, the release of VOCs may be a good indicator of the plant's capacity to activate jasmonate-inducible direct defences, like, for example, hydroxamic acids or proteases (Erb *et al*. 2009b; Shivaji *et al*. 2010). The importance of the upstream signalling events preceding volatile release in the leaves is also illustrated by the fact that the quantities of many HIPVs showed positive correlations, even when they were synthesized via different biochemical pathways (Table 3). In nature, HIPVs may therefore be used as cues to assess a plant's defensive state as well as its induced resistance capacity. Lepidopteran and aphid herbivores are repelled by inducible VOCs (Bernasconi *et al*. 1998; De Moraes, Mescher & Tumlinson 2001). However, coleopteran herbivores tend to be attracted to HIPVs (Landolt, Tumlinson & Alborn 1999; Fernandez & Hilker 2007) and it has been reported that the release of VOCs by transgenic tobacco plants increases colonization by herbivores in the field (Halitschke *et al*. 2008). In the specific case of maize, certain VOCs are known to attract the larvae of the moth *Spodoptera frugiperda* (Carroll *et al*. 2006). It remains to be determined if HIPVs can benefit maize plants by signalling a strong defence capacity, or if the costs associated with a facilitation of host location by herbivores outweigh this effect. As HIPVs are relatively simple and quick to measure, HIPV-based marker-assisted selection procedures could be developed to breed for insect resistance in maize.

We found no correlation between leaf and root herbivore resistance or between the resistance against the two pathogens, but the plant's capacity to withstand the northern corn

Table 3. Correlation coefficients (Pearson product moment correlations) between 21 maize genotypes for the different induced leaf volatile compounds

	Compound	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25
1	(*E*)-2-hexenal	**0.50**	0.00	0.47	**0.56**	0.21	0.32	0.25	0.24	0.10	0.02	–0.11	0.22	–0.13	0.10	0.05	0.39	0.15	0.01	–0.34	–0.06	0.05	0.06	0.35
2	(*Z*)-3-hexen-1-ol		–0.34	**0.75**	0.37	**0.57**	0.32	0.06	0.11	0.33	0.07	0.13	–0.21	0.01	0.31	0.27	0.10	0.33	–0.41	–0.15	0.03	0.11	0.09	**0.46**
3	*β*-myrcene			–0.18	0.34	–0.20	–0.08	–0.14	–0.21	0.22	0.05	–0.06	0.05	–0.16	–0.02	–0.14	–0.15	–0.11	–0.05	–0.23	–0.18	0.01	–0.23	–0.04
4	(*Z*)-3-hexenyl acetate				**0.69**	**0.53**	0.27	0.13	0.07	0.15	0.21	0.15	–0.12	0.11	**0.48**	**0.63**	0.20	**0.58**	–0.33	–0.20	–0.19	0.18	0.38	**0.49**
5	Linalool					0.42	0.36	0.03	0.23	0.17	0.40	0.30	0.13	–0.04	0.33	**0.49**	0.17	**0.52**	0.05	–0.43	–0.22	0.08	0.31	0.54
6	DMTT						**0.47**	–0.24	0.27	0.13	0.43	**0.45**	–0.17	–0.31	0.07	**0.46**	–0.26	0.39	–0.10	–0.02	0.11	–0.18	0.12	**0.67**
7	2-phenthenyl acetate							0.22	**0.77**	–0.07	0.16	–0.02	–0.22	–0.17	0.18	0.22	0.20	0.26	0.10	–0.27	0.21	0.16	0.28	0.37
8	Methyl antranilate								**0.51**	–0.09	0.12	–0.15	–0.09	0.27	**0.68**	–0.05	**0.89**	**0.47**	–0.15	–0.09	0.11	**0.80**	0.43	–0.19
9	Indole									–0.13	0.21	0.02	–0.04	0.10	0.34	0.09	**0.44**	**0.47**	0.37	–0.10	**0.51**	0.37	**0.52**	0.08
10	Gernanyl acetate										–0.27	–0.22	–0.17	–0.12	0.25	–0.09	–0.13	0.04	–0.28	–0.17	–0.10	0.25	–0.27	–0.08
11	Cycloisosativene^{N}											**0.90**	0.00	–0.22	0.03	0.35	0.15	**0.47**	–0.01	–0.12	–0.10	–0.14	0.41	**0.53**
12	*α*-ylangene^{N}												0.04	–0.28	–0.12	0.40	–0.12	0.35	0.00	–0.03	–0.08	–0.34	0.30	**0.59**
13	(*E*)-*α*-copaene													0.12	–0.26	–0.09	0.15	–0.22	**0.72**	0.43	0.37	–0.13	0.15	–0.11
14	(*E*)-*β*-caryophyllene														0.41	–0.14	0.24	0.29	0.27	0.28	0.16	0.37	0.36	–0.34
15	(*E*)-*α*-bergamotene															0.16	**0.53**	**0.77**	–0.30	–0.06	–0.03	**0.88**	0.42	–0.09
16	Sesquiterpene^{N}																–0.07	**0.48**	–0.15	–0.09	–0.10	–0.12	**0.58**	**0.66**
17	*α*-humulene																	0.32	–0.03	–0.12	0.06	**0.67**	0.32	–0.15
18	(*E*)-*β*-farnesene																		–0.11	–0.09	0.07	0.46	**0.71**	0.31
20	Germacrene D																			0.30	**0.47**	–0.20	0.25	–0.16
21	Sesquiterpene II^{N}																				**0.51**	0.03	0.22	–0.27
22	(*E*,*E*)-*α*-farnesene																					0.05	0.33	–0.12
23	*β*-sesquiphellandrene																						0.27	–0.38
24	Nerolidol																							0.24
25	TMTT	^{N}tentative identification																						

Bold numbers indicate a significant correlation ($P < 0.05$).
N, tentative identification.

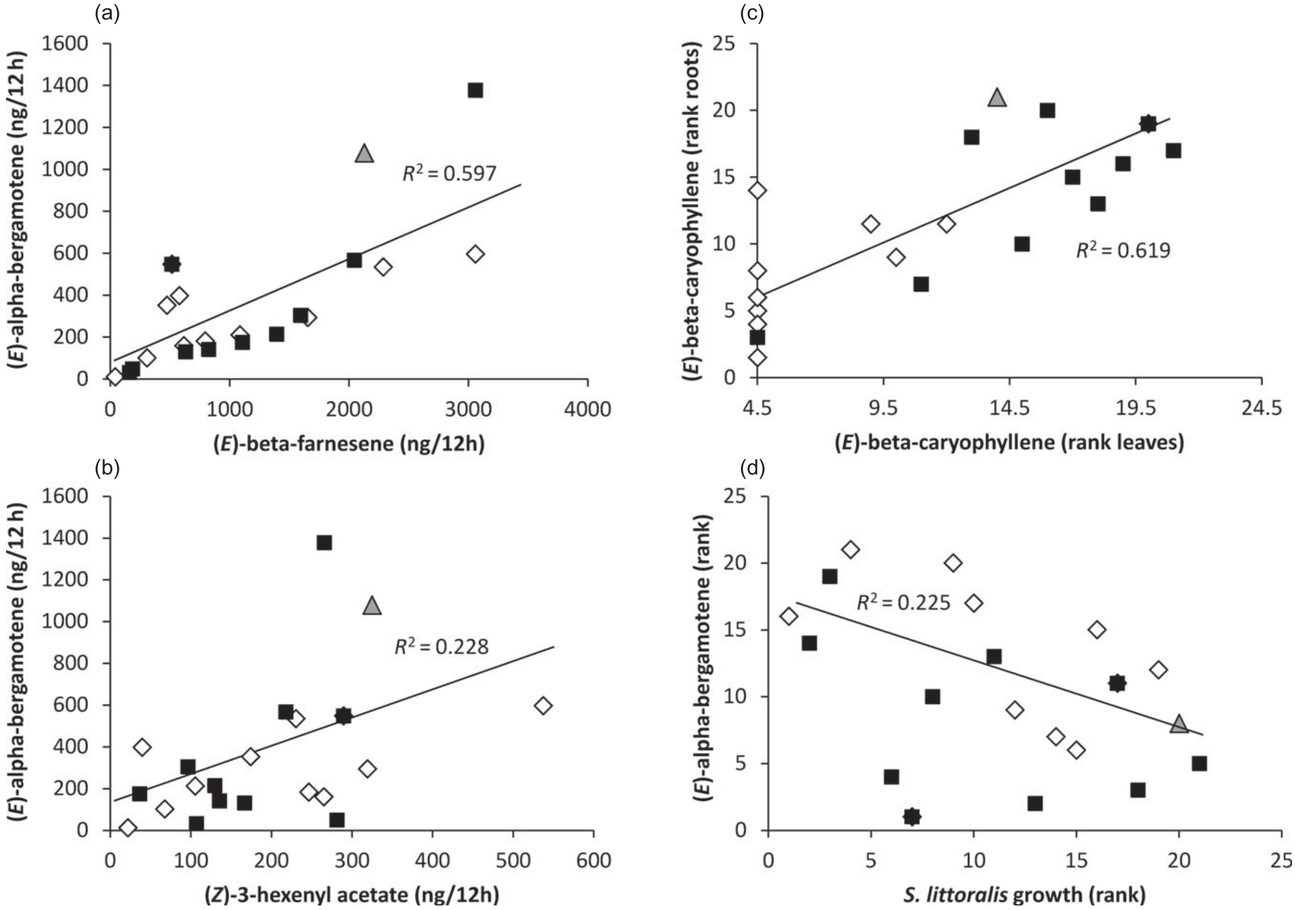

Figure 6. Correlations of leaf volatiles. White symbols: dent lines; black symbols: flint lines; grey symbols: Delprim (hybrid). (a) Correlation between the emission of (*E*)-*α*-bergamotene and (*E*)-*β*-farnesene in the different genotypes. (b) Correlation between the emission of (*E*)-*α*-bergamotene and (*Z*)-3-hexenyl acetate in the different genotypes. (c) Correlation of induced (*E*)-*β*-caryophyllene emission in the leaves and the roots. (d) Correlation of the induced emission of (*E*)-*β*-farnesene and the growth of *S. littoralis*. Genotypes are ranked from 1 (lowest emission, weakest herbivore growth) to 21 (highest emission, strongest herbivore growth). Linear regression lines and R^2 goodness-of-fit values are shown.

leaf blight, *S. turcica* (Fig. 2a), showed a negative correlation with herbivore resistance (Fig. 2d). Genotypes that were of poor quality for the herbivore supported stronger growth of the fungus and *vice versa*. In a previous study, we have shown that the transcriptional responses of maize plants to infestation by *S. turcica* and *S. littoralis* differ markedly (Erb *et al*. 2009a). The results presented here confirm that different mechanisms determine the performance of these two attackers. There is considerable interest in trade-offs between insect and pathogen resistance (Felton & Korth 2000; Dicke, van Loon & Soler 2009), but most studies have focused on multiple attacker situations (De Vos *et al*. 2006; Van Oosten *et al*. 2008), and little information is available on genetic trade-offs in single-attacker situations. This is surprising, given their obvious ecological and agricultural importance: From an ecological point of view, trade-offs between the molecular mechanisms would mean that wild plants are unlikely to reach full resistance against both insects and pathogens, which may lead to population fluctuations and, possibly, diffuse co-evolution (Strauss *et al*. 2005). For plant breeders, our results imply that stacking of several resistance parameters may not always be possible, as they may constrain each other. Further research will aim at disentangling the mechanism behind the genetic trade-offs between herbivore and pathogen resistance in maize.

The comparison of the inbred lines provides insights into the intraspecific variation in root volatile release of maize plants. Root HIPVs are important signals in belowground tritrophic interactions (Neveu *et al*. 2002; Rasmann *et al*. 2005; Ali, Alborn & Stelinski 2010), and knowledge about their diversity may help to optimize pest control strategies. Previous research has shown that maize plants predominantly release the sesquiterpene (*E*)-*β*-caryophyllene after *D. virgifera* attack, while the volatile bouquet of cotton after infestation by the same herbivore is more complex (Rasmann & Turlings 2008). Here, we demonstrate that treatment with JA induces at least 12 different VOCs in maize roots, including sesquiterpenes, alkanes and aldehydes as well as traces of indole (Table 1). Sesquiterpenes could be separated into products of at least two terpene synthases: TPS23, which produces (*E*)-*β*-caryophyllene and *α*-humulene (Köllner *et al*. 2008), and TPS8, which is responsible for the production of *α*-copaene and (*E*)-*α*-cubebene. Based on their correlation coefficients (Table 2) and the PCA (Fig. 4d), it seems likely that the two unknown sesquiterpenes are products of TPS8 as well. It remains to be determined how similar the JA and *D. virgifera* induced blends are. It is possible that JA induction only partially mimics the signalling events following root herbivory, and additional genetic links may be discovered using a treatment that simulates herbivore attack more closely. Unfortunately, no *D. virgifera* elicitors are known, and inflicting artificial damage to the roots remains a challenge.

Similar to what can be observed for the leaves (Degen *et al*. 2004), the maize genotypes differ markedly in their capacity to produce root VOCs (Fig. 3). We found negative correlations between the production of alkanes/aldehydes and a number of sesquiterpenes (Table 2), suggesting that, in contrast to the leaves, different mechanisms are responsible for their induction in the roots, and that there may be constraints regarding their production. Their physiological and ecological importance remains to be determined. (*E*)-*β*-caryophyllene, the main signal that attracts entomopathogenic nematodes (Rasmann *et al*. 2005), was produced by induced leaves as well as roots, and our results show a close positive semi-quantitative relationship between leaf and root emission (Fig. 6c). This suggests that the upstream signals that are responsible for the induction of (*E*)-*β*-caryophyllene are at least partially overlapping and that a plant's capacity to produce the belowground signal can be estimated by measuring induced leaf emissions. It should be noted that we used a destructive technique to assess root volatile emission. Even though this method is well established (Rasmann *et al*. 2005) and it is expected that inducible terpenoids are rapidly released upon synthesis (Köllner *et al*. 2008), it is possible that some of the measured VOCs are not actually released by the plant but are stored in the cells. Further research aims at assessing root volatile release *in vivo*.

Our study also enabled us to evaluate if the difference in breeding history led to divergent resistance phenotypes. Dent corn (*Z. mays* indentata) is mostly grown in North America, while flint lines (*Z. mays* indurate) are frequently used in Asia and Europe (Acquaah 2007). Historically, flint lines were grown in the southwestern USA and Mexico for up to 3000 years, and were replaced by early dent cultivars 500 years ago (Troyer 1999). Flint lines are considered to be better adapted to longer days and cooler climatic conditions, and have therefore played a key role in the early introduction of maize to Europe at the end of the 15th century (Rebourg *et al*. 2003). We found that flint and dent lines differ in a number of resistance parameters. Dent lines were more tolerant to root herbivory by *D. virgifera* (Fig. 1d), and their resistance to *C. graminicola* was similarly high (Fig. 1b). On the other hand, flint lines produced higher amounts of root sesquiterpenes, including (*E*)-*β*-caryophyllene. This is in accordance with earlier findings that many North American lines have lost their capacity to produce this signal (Degen *et al*. 2004; Rasmann *et al*. 2005; Köllner *et al*. 2008). We report here that several typical leaf volatiles like indole and germacrene D are also emitted in lower amounts by dent lines. Our results suggest that dent lines may have retained a higher overall potential to resist pathogens and insects, but may have lost some of the key signals to recruit organisms of the third trophic level.

CONCLUSIONS

By studying insect and pathogen resistance in 21 different maize genotypes, we demonstrate that genetic links exist between different defensive strategies in plants. We show that insect resistance is positively correlated with the release of inducible volatiles, but that it displays a trade-off with pathogen resistance. Several defensive traits, including resistance to two different pathogens as well as root and leaf herbivory, did not show any apparent correlation, suggesting independent regulation. We conclude that the genetic make-up of plants can lead to both positive and negative interactions between defensive traits in some cases, even when only a single attacker is present at a given time. Such synergies and trade-offs may shape the evolution of plants and are important to consider when breeding for resistance in crop plants.

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