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Intraspecific variation in herbivore community composition and transcriptional profiles in field-grown *Brassica oleracea* cultivars

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Abstract

Intraspecific differences in plant defence traits are often correlated with variation in transcriptional profiles and can affect the composition of herbivore communities on field-grown plants. However, most studies on transcriptional profiling of plant–herbivore interactions have been carried out under controlled conditions in the laboratory or greenhouse and only a few examine intraspecific transcriptional variation. Here, intraspecific variation in herbivore community composition and transcriptional profiles between two *Brassica oleracea* cultivars grown in the field is addressed. Early in the season, no differences in community composition were found for naturally occurring herbivores, whereas cultivars differed greatly in abundance, species richness, and herbivore community later in the season. Genome-wide transcriptomic analysis using an *Arabidopsis thaliana* oligonucleotide microarray showed clear differences for the expression levels of 26 genes between the two cultivars later in the season. Several defence-related genes showed higher levels of expression in the cultivar that harboured the lowest numbers of herbivores. Our study shows that herbivore community composition develops differentially throughout the season on the two *B. oleracea* cultivars grown in the field. The correlation between the differences in herbivore communities and differential expression of particular defence-related genes is discussed.

Key words: *Brassica oleracea*, comparative genomics, gene expression, herbivorous insects, microarray, species richness.

Introduction

Intraspecific variation in plant traits may influence the composition and diversity of herbivore communities on plants grown under natural conditions (Wimp *et al.*, 2005; Whitham *et al.*, 2006; Poelman *et al.*, 2009). Plant traits that affect herbivores include morphological factors, such as wax layers, and defence-related secondary metabolites (Schoonhoven *et al.*, 2005). These plant traits can be constitutively present, but plants can also alter their phenotype in response to herbivory (Kessler and Baldwin, 2002; Howe and Jander, 2008; Inbar and Gerling, 2008; Karban, 2008). Differences in the transcription of particular genes have been shown to correlate with intraspecific variation in phenotypic traits (Carroll, 2000). Studies on different populations of the same species have revealed that variation in transcription of defence-related genes is responsible for variation in secondary metabolite production (Wu *et al.*, 2008) and herbivore resistance (Kuśnierczyk *et al.*, 2007; Gao *et al.*, 2008). Furthermore, the expression of genes regulating the biosynthesis of jasmonic acid, a plant hormone known to mediate plant defences, has been shown significantly to affect the composition of the herbivore community on tobacco plants (Kessler and Baldwin, 2004). However, no data are available from field studies that link intraspecific variation in plant gene expression with herbivore community composition.
Intraspecific variation in herbivore community composition can be affected by induced plant responses. In *Brassica oleracea* plants, for example, experimentally introducing *Pieris rapae* caterpillars early in the season influenced herbivore community composition later in the season (Poelman et al., 2008; EH Poelman, JJA Van Loon, NM Van Dam, LEM Vet, M Dicke, unpublished data). Depending on their feeding strategy herbivores differentially induce plant responses (Walling, 2000), which may affect the performance of the initial herbivore as well as that of subsequently colonizing species (Agrawal, 2000; Traw and Dawson, 2002). Induced plant responses not only influence the performance of subsequent herbivores feeding on the plant, but may also affect their host plant preference (De Moraes et al., 2001; Shiojiri et al., 2002; Long et al., 2007; EH Poelman, JJA Van Loon, NM Van Dam, LEM Vet, M Dicke, unpublished data).

Herbivores may be differentially affected by induced plant responses depending on their degree of host plant specialization. Induced secondary metabolites that have a negative effect on generalist herbivores may act as feeding stimulants or can be detoxified by specialists (Agrawal, 2000; Ratzka et al., 2002; Wittstock et al., 2004; Kliebenstein et al., 2005; Després et al., 2007). Specialists may even be able to accumulate certain defence-related secondary metabolites to use them for their own defence (Després et al., 2007; Kazana et al., 2007). However, it should be noted that specialists may still be susceptible to the toxic effects of secondary metabolites (Adler et al., 1995; Agrawal and Kurashedge, 2003; Steppuhn et al., 2004; Ballhorn et al., 2007). Differences between generalists and specialists can also be found with regard to attraction: generalist herbivores often avoid induced plants, whereas some specialists may prefer these plants (Bolter et al., 1997; Kaplan and Denno, 2007; Long et al., 2007; Poelman et al., 2008).

Signal transduction pathways underlie induced defences in which the plant hormones jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) play important roles (Kessler and Baldwin, 2002; Pieterse and Dicke, 2007). Gene expression profiling using microarrays has been profoundly useful in investigating mechanisms of induced defences (Rishi et al., 2002; Hui et al., 2003; Korth, 2003; Reymond et al., 2004; Voelckel and Baldwin, 2004; De Vos et al., 2005; Thompson and Goggin, 2006; Smith and Boyko, 2007). Most of these studies have been performed under carefully controlled environmental conditions in the greenhouse in which plants are exposed to a single attacker. In natural habitats, however, plants can be exposed to multiple herbivores simultaneously and under a variety of conditions. It is unclear whether differences in gene expression observed in the greenhouse are sustained in the field. Transcriptional responses in field-grown plants have been studied after exposure to methyl jasmonate (Schmidt and Baldwin, 2006), induction by *Manduca sexta* herbivory (Izaguirre et al., 2003), or Japanese beetles (*Popillia japonica*) (Casteel et al., 2008). None of these studies have investigated intraspecific variation in gene expression nor did they monitor the presence of naturally occurring herbivorous insects.

In the Brassicaceae, domestication has given rise to several important crops including white cabbage (*B. oleracea* var. *capitata*). As many varieties are available and plants within a variety are quite uniform, *B. oleracea* cultivars provide a unique opportunity to investigate intraspecific patterns of gene expression in response to herbivory. Intraspecific variation in the secondary metabolite content of four *B. oleracea* var. *capitata* cultivars (Rivera, Lennox, Christmas Drumhead, and Badger Shipper) has been shown to influence herbivore community composition in the field (Poelman et al., 2009). Two of these cultivars, Rivera and Christmas Drumhead, have also been shown to differ in transcriptional responses to herbivory by caterpillars of the Small Cabbage White *Pieris rapae* and the cabbage aphid *Brevicoryne brassicae* feeding under greenhouse conditions (Broekgaarden et al., 2007, 2008). The present study addresses the question whether differences in herbivore community composition in the field between two cultivars from the same species, i.e. the two *B. oleracea* cultivars Rivera and Christmas Drumhead, can be related to intraspecific variation in gene expression. To our knowledge, this is the first study that links herbivore community dynamics and whole-genome gene expression under field conditions where plants are exposed to naturally occurring herbivores.

**Materials and methods**

**Plant growth**

Seeds of the F2 hybrid white cabbage (*Brassica oleracea* var. *capitata*) cultivar Rivera and the open-pollinated cultivar Christmas Drumhead were obtained from Bejo Zaden BV (Warmenhuizen, The Netherlands) and the Centre of Genetic Resources, The Netherlands (CGN), respectively. Seeds were directly sown in peat soil cubes containing potting compost (Lentse Potgrond BV, The Netherlands) and allowed to germinate in a greenhouse compartment (22–26°C light/dark; 40–70% relative humidity). Prior to being transplanted into the field site, trays with peat soil cubes containing 3-week-old seedlings were placed outside the greenhouse during the day for 2 weeks. Both cultivars show similar morphological and growth characteristics (see Supplementary Fig. S1 at *JXB* online). Christmas Drumhead is somewhat earlier in forming a head than Rivera.

**Field site**

In 2007, a field experiment in an agricultural field near Wageningen, The Netherlands was established. Eighteen plots (6×6 m) with a monoculture of one of the two cultivars (ten plots for Rivera and eight plots for Christmas Drumhead) were established using a randomized design. Five-week-old plants were transferred with their peat soil cubes to the field in week 19 (7 May) of 2007. Plots contained 49 plants in a square of 7×7 plants with a spacing of 75 cm between plants. A strip of 6 m sown with a grass mixture of *Lolium* and *Poa* species isolated the plots.

**Collection of material**

In week 23 (6 June) and week 32 (6 August), i.e. 4 weeks and 13 weeks after plants had been transferred to the field, respectively, material was collected from 18 plots (ten for Rivera and eight for Christmas Drumhead). The two time points were selected based on peaks in the herbivore abundance in 2005 (Poelman et al., 2009) and 2006 (EH Poelman, JJA Van Loon, NM Van Dam, LEM Vet, M Dicke, unpublished data). One leaf disc (diameter 2.3 cm) was
harvested from a young leaf of nine separate plants in each plot, and the leaf discs were pooled to create a single sample per plot. Upon harvesting, samples were immediately flash-frozen in liquid nitrogen and stored at -80 °C. After collecting leaf discs, the same plants were completely harvested in plastic bags to monitor the presence of naturally occurring insects. Bags were stored at 4 °C until plants were monitored. All plants were monitored within 5 d. To assess whether plant biomass or the number of leaves could explain the differences in herbivore community composition between the cultivars, all plants were weighed individually and the number of leaves per plant was counted.

Herbivore biodiversity calculations and analysis

For both time points, the number of individuals per herbivore species was counted on the nine plants of a plot and herbivores were weighed on a microgram balance. These values were used to calculate per plant (i) the total herbivore abundance, (ii) the species richness, and (iii) the Shannon-Wiener diversity index. Total herbivore abundance represents the total number of individuals, whereas species richness represents the total number of herbivorous species. The Shannon-Wiener biodiversity index describes herbivore diversity by taking into account both the richness of species as well as the evenness of their distribution (Mendes et al., 2008). Both a large number of unique species and higher evenness of their abundance distribution increase the value of this index. Differences between the two cultivars for all measured parameters were statistically analysed with Mann–Whitney U tests.

Herbivore abundance, species richness, total herbivore mass, and biodiversity index were regressed onto plant weight and number of leaves in multiple linear regression analysis, with plant biomass and biodiversity index were regressed onto plant weight and number of leaves in multiple linear regression analysis, with cultivar as the grouping factor.

RNA isolation, aRNA synthesis, and dye labelling

Leaf samples from two plots were pooled per cultivar and three biological replicates were analysed per cultivar. Total RNA was extracted with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) followed by a purification using the RNeasy Plant Mini kit (Qiagen, Valencia, CA, USA). Four micrograms of total RNA were linearly amplified using the Amino Allyl MessageAmp II aRNA Amplification kit (Ambion, Austin, TX, USA). Samples were labelled with Cy3 (Rivera) and Cy5 (Christmas Drumhead) monoreactive dye (Amersham, Piscataway, NY, USA). Amplified RNA (aRNA) was labelled in freshly made 0.2 M sodium carbonate buffer (pH 9.0) for 1 h at room temperature. Dye incorporation was monitored by measuring the Cy3 and Cy5 fluorescence emissions using a nanodrop ND-1000 UV-Vis Spectrophotometer (Bio-Rad, Hercules, CA, USA).

70-mer A. thaliana microarray

Microarrays containing 70-mer oligonucleotides, based on the genome of A. thaliana, were obtained from the group of David Galbraith from the University of Arizona, Tucson, AZ, USA (http://www.ag.arizona.edu/microarray). These microarrays contain 29 110 probes from the Operon Arabidopsis Genome Oligo Set Version 3.0 (Operon). This oligo set represents 26 173 protein-coding genes, 28 964 protein-coding gene transcripts, and 87 miRNAs. The majority of genes are represented by one 70-mer probe on the microarray. A 70-mer instead of a 25-mer microarray was used as the longer oligos have a higher sensitivity (Relogio et al., 2002) and non-specific binding of mismatched targets can be kept to a minimum by using long probes (Buckley, 2007). Moreover, the microarray has proved to be a good tool to study transcriptomics in B. oleracea (Lee et al., 2004; Broekgaarden et al., 2007, 2008; Fatouros et al., 2008). In addition, independent support for this approach is provided by other studies involving heterologous hybridizations (Becher et al., 2004; Buckley, 2007; Davey et al., 2009). Combined data obtained from 72 hybridizations using B. oleracea material (data from Broekgaarden et al., 2007, 2008; data presented in this manuscript; C Broekgaarden, unpublished data) show that around 90% of the oligonucleotides present on the microarray hybridized in at least two experiments. Although the two cultivars used in this study are from the same species, the overlap in hybridizing probes was not complete, and around 80% of the probes hybridized with material from each cultivar separately.

It should be realized that the transcription of genes that are specific for B. oleracea will not be detected with A. thaliana microarrays. Yet, the use of the 70-mer A. thaliana microarray provides a good tool to investigate transcriptomic changes of a large proportion of B. oleracea genes, which is a great advantage of this approach.

Microarray hybridization

Immobilization of the array elements was performed according to the manufacturer’s website. The arrays used all originated from the same printing batch, thus eliminating batch to batch variation. The hybridization mixture contained 100 pmol of the Cy3-labelled sample, 50 pmol of the Cy5-labelled sample, 2× SSC, 0.08% SDS, and 4.8 μL Liquid Block (Amersham) in a final volume of 80 μL. The solution was incubated at 65 °C for 5 min before applying to the microarray covered with a lifterslip (Gerhard Menzel, Braunschweig, Germany). The microarray was placed in a hybridization chamber (Genetix, New Milton, Hampshire, UK) and incubated at 50 °C. After 12 h the microarray was washed for 5 min in 2× SSC/0.5% SDS at 50 °C, followed by a 5 min wash in 0.5× SSC at room temperature, and a final 5 min wash in 0.05× SSC at room temperature. The microarray was immediately dried by centrifugation for 4 min at 200 rpm.

Hybridized microarrays were scanned with a ScanArray Express HT Scanner (PerkinElmer, Waltham, MA, USA).

Microarray analysis

Mean fluorescent intensities for Cy3 and Cy5 were determined using the ScanArray Express software (PerkinElmer). Each image was overlaid with a grid to assess the signal intensities for both dyes from each spot. Background fluorescence was subtracted and spots with adjusted intensities lower than half the background were manually raised to half the background to avoid extreme expression ratios. Spots were excluded from the analysis when: (i) showing signal intensities less than half the background for both dyes; (ii) showing aberrant shape; or (iii) located in a smear of fluorescence. To correct for hybridization efficiency differences between the cultivars, spots that have been shown to hybridize with material from one cultivar only were also removed from the data analysis. Lowess (loccit) normalization was carried out within each slide using TIGR MIDAS version 2.19 (The Institute for Genomic Research, Rockville, MD, USA) to remove any systematic dye effects, assuming that the overall gene expression for the two cultivars is approximately equal. Normalized expression ratios for each individual spot and the mean of the three replicate spots were calculated. A Student’s t test on log2 transformed expression ratios was conducted for each experimental condition using TIGR MEV version 3.1. To address the issue of multiple testing and to identify the proportion of false positives among the genes identified as differentially expressed, the false discovery rate (FDR) was calculated using the Benjamini–Hochberg method (Benjamini and Hochberg, 1995). A q-value was computed for each gene with a log2 expression ratio >1 or ≤-1 using the distribution of P values of all measurements. Genes that showed a significant difference in expression level (P <0.05 and FDR <0.1) and a log2 expression ratio >1 or ≤-1 were considered to be higher expressed in Rivera or Christmas Drumhead, respectively. The names of A. thaliana homologues were used to identify B. oleracea genes and examined the potential function of differentially regulated genes according to gene ontology (GO) terms from The Arabidopsis Information Resource (http://www.arabidopsis.org).
Quantitative RT-PCR
Quantitative RT-PCR was used to examine gene expression of selected genes per plot by using the RNA pools of all 18 plots separately. One microgram of total RNA was treated with DNase I (Invitrogen) according to the manufacturer’s instructions. DNA-free total RNA was converted into cDNA using the iScript cDNA synthesis kit (Bio-Rad). Gene-specific primers were designed for B. oleracea genes based on sequences obtained by a BLAST search in the TIGR B. oleracea database (Lipoxygenase 2, LOX2; left 5'-CTT TGC TCA CAT ACG GTA GAA GC-3', right 5'-CCT TGT CAT TGG GCT AGT TC-3'; Trypsin-and-protease inhibitor, TPI; left 5'-TGG TGA CAA GTA GCT GTG GTC-3', right 5'-TCC AAG TTA GGA GTG G-3'). Primers were tested for gene specificity by performing melt curve analysis and PCR products were sequenced to confirm amplification of the gene of interest. Sequence results were checked by a BLAST search in the TIGR database as well as in the A. thaliana TIGR database. Quantitative RT-PCR analysis was done in optical 96-well plates with a MyIQ Single-Color Real-Time PCR Detection System (Bio-Rad), using SYBR Green to monitor dsDNA synthesis. Each reaction contained 10 µl 2× SYBR Green Supermix Reagent (Bio-Rad), 10 ng cDNA, and 300 nM of each gene-specific primer in a final volume of 20 µl. All qRT-PCR were performed in duplicate and average values were used in the analyses. The following PCR program was used for all PCR reactions: 3 min at 95 °C; 40 cycles of 30 s at 95 °C and 45 s at 60 °C. Threshold cycle (Ct) values were calculated using Optical System software, version 2.0 for MyIQ (Bio-Rad). Subsequently, Ct values were normalized for differences in cDNA synthesis by subtracting the Ct value of the constitutively expressed gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH: left 5'-AGA GCC GCT TCC TTC AAC ATC ATT-3', right 5'-TGG GCA CAC GGA AGG ACA TAC C-3') from the Ct value of the gene of interest. Normalized gene expression was then calculated as 2^{-ΔΔCt}. Differences between time points were analysed with one-way analysis of variance (ANOVA) followed by Post Hoc multiple comparison tests with Least Significant Difference (LSD).

Results
Abundance of naturally occurring herbivores
Fourteen species of herbivorous insects were found in the field (Table 1), all of which were previously reported to be associated with B. oleracea (Root, 1973; Mitchell and Richards, 1979; Poelman et al., 2009). Thirteen occurred on both cultivars and one, Autographa gamma, was only found on Christmas Drumhead. Early in the season, 4 weeks after transplanting seedlings into the field, nine herbivore species were found on Rivera and Christmas Drumhead with no differences in abundance on the two cultivars (Fig. 1). At this point in the season, Brevicoryne brassicae was the most abundant herbivore on both cultivars with about 20 individuals per plant.

Nine weeks after the first time point, the abundance and species richness of herbivores on the cultivars had changed completely and was lower on Rivera than on Christmas Drumhead (Mann–Whitney U test, abundance: U=0, P <0.001; richness: U=2, P <0.001). Rivera harboured significantly fewer Pieris rapae and Mamestra brassicae larvae than Christmas Drumhead (Mann–Whitney U test, P. rapae: U=0, P <0.001; M. brassicae: U=5, P=0.001; Fig. 1). Several larvae of A. gamma were found on Christmas Drumhead, whereas this species was absent on Rivera (Fig. 1). Furthermore, less than half as many flea beetles were found on Rivera than on Christmas Drumhead (Phyllotreta atra: U=7, P=0.002; Phyllotreta undulata: U=12, P=0.012; Fig. 1). Large differences between the cultivars were found for the occurrence of cabbage aphids (B. brassicae U=0, P <0.001) and whiteflies (Aleyrodites proletella U=0, P <0.001) later in the season. Hardly any individuals of these two species were present on Rivera, whereas on Christmas Drumhead c. 30 and 70 individuals per plant were found of these two species, respectively (Fig. 1). Remarkably, a few A. proletella adults were found on Rivera, but no pupae of this species were present on this cultivar. Four times more A. proletella pupae than adults were found on Christmas Drumhead. In addition, lower numbers of the phloem-feeding herbivore Myzus persicae were found on Rivera than on Christmas Drumhead (U=0, P <0.001; Fig. 1). Three species of lepidopteran larvae were equally distributed over the two cultivars (P. brassicae: U=29, P=0.360; Plutella xylostella: U=21, P=0.101; Evergestis forficatis: U=25, P=0.203; Fig. 1).

In general, Rivera harboured significantly lower abundance and species richness of specialist (abundance: U=0,
Herbivore abundance, species richness, total herbivore mass, or biodiversity were not significantly affected by plant weight or number of leaves (linear regression df = 15, abundance: weight P = 0.96, leaves P = 0.17; richness: weight P = 0.74, leaves P = 1.00; mass: weight P = 0.43, leaves P = 0.47; Shannon–Wiener index: weight P = 0.50, leaves P = 0.84).

**Gene expression differences between Rivera and Christmas Drumhead in the field**

*Arabidopsis thaliana* full-genome microarrays were used to test whether differences in gene expression levels exist between the two cultivars under field conditions. Early in the season, only a small number of genes showed significantly different expression levels between the two cultivars. One and five genes showed higher levels of expression in Rivera or Christmas Drumhead, respectively, including genes mainly involved in general metabolic processes and genes of unknown function (Table 2). Later in the season, differences in expression levels between Rivera and Christmas Drumhead were more pronounced as 26 genes showed different expression levels (Table 2). The 12 genes with higher expression levels in Rivera include, among others, genes involved in defence and metabolic processes. The defence-related genes that were identified in Rivera include genes encoding lipoygenase 2 (LOX2), lectin (At2g39310), a trypsin inhibitor, and a Bet v I allergen (Table 2). In Christmas Drumhead, 14 genes showed higher expression levels than in Rivera of which most were involved in metabolic processes (Table 2). One of the genes with a higher expression level in this cultivar is involved in defence and encodes flavin-dependent monooxygenase 1 (FMO1) (Table 2).

**Gene expression in the field compared to herbivore-induced responses in the greenhouse**

The genes that showed different levels of expression in field-grown Rivera and Christmas Drumhead plants were compared to previously identified *P. rapae*- and *B. brassicae*-induced genes in plants grown in the greenhouse (Broekgaarden *et al.*, 2007, 2008). About half (5/12) of the genes that showed a higher level of expression in Rivera compared to Christmas Drumhead in the field were previously identified as *P. rapae*-inducible in one or both cultivars in the greenhouse (Broekgaarden *et al.*, 2007; Fig. 3), including the defence-related genes LOX2, trypsin inhibitor, and the gene encoding lectin (At2g39310). Only one of the 14 genes, transcription factor B3, that showed...
a higher expression level in Christmas Drumhead compared to Rivera in the field, were previously identified as *P. rapae*-inducible in one or both cultivars under greenhouse conditions (Broekgaarden *et al.*, 2007; Fig. 3). None of the genes that showed a differential expression between the cultivars under field conditions were previously identified as *B. brassicae*-responsive (Broekgaarden *et al.*, 2008).

Quantitative RT-PCR analysis using *B. oleracea*-derived primers for *LOX2*, a gene known to be involved in defence, confirmed the microarray result by showing a significantly higher expression in Rivera (1.30±0.09) than in Christmas Drumhead (0.52±0.13) in the field (independent sample *t* test, *P* <0.001). In order to compare gene expression levels between field- and greenhouse-grown plants, absolute gene expression levels were calculated from data obtained from control plants and *P. rapae*-challenged plants of Rivera and Christmas Drumhead grown in the greenhouse (Broekgaarden *et al.*, 2007). Expression levels of *LOX2* in field-grown plants were significantly higher than those in control plants grown in the greenhouse for both cultivars (one-way ANOVA, df=4, *P* <0.001; Fig. 4). However, expression levels in the field were significantly lower than the levels reached after 72 h of *P. rapae* feeding for Christmas Drumhead (*P* =0.048; Fig. 4).

The expression of *TPI* (*trypsin-and-protease inhibitor*), a defence-related gene that has been shown to be *P. rapae-* and *B. brassicae*-inducible in the greenhouse (Broekgaarden *et al.*, 2007, 2008) was also monitored. In *A. thaliana*,
disrupting the expression of TPI resulted in better performance of P. rapae and B. brassicae (Broekgaarden et al., 2008) indicating a role for this gene in induced plant defences and therefore in affecting herbivore community composition. The microarray showed that TPI expression levels were 2.61 times higher in Rivera than in Christmas Drumhead, however, due to large variation between the replicates, the difference was not significant (P=0.116; FDR=0.162). QRT-PCR analysis using B. oleracea-derived primers revealed a significantly higher level of expression in Rivera (1.56±0.20) than in Christmas Drumhead (0.93±0.36) for this gene (independent sample t test, P=0.04). For both cultivars, the expression levels of TPI were significantly higher in field-grown plants compared to control plants grown in the greenhouse for both cultivars (one-way ANOVA, df=4, P <0.001; Fig. 5A). However, TPI expression levels in field-grown plants did not reach the levels of greenhouse-grown plants challenged for 48 h or 72 h with P. rapae (Rivera 48 h: P=0.02; Rivera 72 h: P=0.003; Christmas Drumhead 48 h: P=0.01; Christmas Drumhead 72 h: P=0.02; Fig. 5A). By contrast, expression levels of TPI in field-grown plants were significantly higher than expression levels after B. brassicae feeding in the greenhouse (P <0.001; Fig. 5B).

Discussion
Rivera and Christmas Drumhead differentially affect herbivore communities throughout the season

In our field experiment, Rivera and Christmas Drumhead were exposed to naturally occurring populations of herbivorous insects and the abundance of these herbivores was monitored early and later in the season. Early in the season, i.e. 4 weeks after seedlings were planted into the field, the two B. oleracea cultivars harboured similar numbers of herbivorous insects. By contrast, later in the season, when plants were present in the field for 13 weeks, clear differences in herbivore communities were found between the cultivars (Fig. 1). These data show that intraspecific differences in herbivore communities between Rivera and Christmas Drumhead develop throughout the season. This is in agreement with the finding that genotypic differences between plants have a stronger effect on herbivore communities than environmental factors (Bangert et al., 2006; Johnson and Agrawal, 2005).

Since plant size and architecture have been shown to affect insect community composition strongly (Johnson and Agrawal, 2005), the fresh weight and the number of leaves of Rivera and Christmas Drumhead was monitored. Although plants of the two cultivars differed in these two traits, neither of these parameters were correlated with
herbivore abundance, richness, and biodiversity and, therefore, are not likely to explain the observed differences in herbivore communities.

Lower numbers of *P. rapae* and *M. brassicae* larvae were found on Rivera than on Christmas Drumhead later in the season, suggesting differences in larval performance and/or oviposition preference between the cultivars. Indeed, under greenhouse conditions, *P. rapae* butterflies showed a higher preference for Christmas Drumhead than for Rivera (Poelman et al., 2009) and *P. rapae* larvae performed better when feeding on Christmas Drumhead compared to Rivera (Broekgaarden et al., 2007; Poelman et al., 2009). Larvae of *M. brassicae* also performed better on Christmas Drumhead than on Rivera under greenhouse conditions (Poelman et al., 2009).

Induced plant responses may not only affect the performance and host plant selection behaviour of the attacking herbivore, but also that of subsequently colonizing species (Shiojiri et al., 2002; Long et al., 2007). Initial infestations with *P. rapae* on Rivera negatively affected the performance of subsequently colonizing *P. rapae* and *M. brassicae*, as well as the preference of adult females from the latter species (Poelman et al., 2008).

Some remarkably large differences between Rivera and Christmas Drumhead were found for some of the herbivorous species. Several *A. gamma* larvae were found on Christmas Drumhead whereas this species was absent on Rivera, suggesting that butterflies of this species have a strong preference for Christmas Drumhead. This species does not completely avoid Rivera as *A. gamma* caterpillars were found on this cultivar in two previous years (Poelman et al., 2009; EH Poelman, JJA Van Loon, NM Van Dam, LEM Vet, M Dicke, unpublished data). Large differences in numbers of specialist whiteflies and aphids between Rivera and Christmas Drumhead were also found, ranging from (almost) zero to tens (>50) per plant, respectively. No pupae of the cabbage whitefly *A. proletella* have been observed on Rivera, whereas high numbers were found on Christmas Drumhead. This suggests a strong difference in host plant selection behaviour of whitefly females. Interestingly, the

Fig. 5. Expression levels of *TPI* in field-grown plants and plants grown in the greenhouse that were either unchallenged (control) or challenged for 24, 48, or 72 h of *P. rapae* (A) or *B. brassicae* (B) feeding. Bars represent mean *TPI* expression levels relative to the reference gene *GAPDH* for Rivera (white bars) and Christmas Drumhead (black bars) with their corresponding standard error. Bars marked with different letters are significantly different (one-way ANOVA, *P* <0.05).
number of *B. brassicae* individuals on Rivera decreased, whereas population size of this aphid increased on Christmas Drumhead throughout the season. Both cultivars started with similar numbers of *B. brassicae* early in the season. In greenhouse experiments, this aphid was previously shown to be able to settle and reproduce on both cultivars (Broekgaarden *et al.*, 2008), indicating that other factors play a role in this decrease in *B. brassicae* numbers under field conditions.

Herbivores not only differ in feeding strategy, but also in their degree of specialization. Specialists feed on one or a few closely related plant species, whereas generalists feed on many different plants (Schoonhoven *et al.*, 2005). Specialists are adapted to host-plant specific characteristics such as defence compounds that are typically harmful to generalist herbivores (Ratzka *et al.*, 2002; Wittstock *et al.*, 2004; Kliebenstein *et al.*, 2005; Després *et al.*, 2007). In *Brassica* species, glucosinolates and their breakdown products stimulate specialists to oviposit and feed while they deter generalists (Renwick *et al.*, 1992, 2006; Van Loon *et al.*, 1992; Riggin-Bucci and Gould, 1996), which is in accordance with the observed higher abundance, species richness, and total herbivore mass of specialists compared to generalists in our study (Fig. 2). In addition, the specialists’ community differed more between the cultivars than that of generalists (Fig. 2), suggesting the differential induction of defence compounds between Rivera and Christmas Drumhead.

**Intraspecific transcriptional variation in relation to differences in herbivore performance and behaviour**

To investigate transcriptional responses that may underlie the observed differences in herbivore community composition it would be very convenient to have the ability to compare gene expression of control plants with that of plants challenged with naturally occurring herbivores. However, the treatments that would be needed to obtain herbivore-free plants in the field, for example, using nets or pesticides, have a direct effect on the study system. Using such controls introduces an unpredictable variable factor and does not contribute positively to the interpretation of the microarray results. Therefore, the ideal controls for the field situation are not available. Instead, to be able to get an impression of gene expression differences in the field, the transcriptional profiles of Rivera and Christmas Drumhead early and later in the season were compared.

Early in the season, no clear differences in gene expression levels could be detected between Rivera and Christmas Drumhead. Only a small number of genes showed differences in expression levels and none of them were related to defensive processes. Conversely, clear differences in gene expression levels between the cultivars were detected later in the season, which is correlated with the different development of herbivore communities throughout the season on the two cultivars. Although a relatively small number of genes showed differences in expression levels between the two cultivars, the genes that were differently expressed are interesting in relation to insect performance.

Later in the season, five defence-related genes showed higher levels of expression in Rivera than in Christmas Drumhead. One of these genes that probably play a central role in shaping the herbivore community is *LOX2*. It is likely that *LOX2* in *B. oleracea* encodes a 13-LOX (Zheng *et al.*, 2007), which is required for the first step in JA biosynthesis (Schaller *et al.*, 2005; Wasternack *et al.*, 2006). In *A. thaliana*, it has been shown that there is a strong correlation between the level of *LOX2* expression and JA production (Spoel *et al.*, 2003). Furthermore, RNA levels of this gene have been shown to increase in *B. oleracea* after JA treatment, wounding, and herbivore feeding (Broekgaarden *et al.*, 2007; Zheng *et al.*, 2007). Therefore, the higher expression level of *LOX2* in Rivera than in Christmas Drumhead suggests that more JA accumulates in Rivera in the field. This is supported by the observation that 37% of the genes with higher expression levels in Rivera than in Christmas Drumhead are JA-responsive (Table 2). The fact that JA mediates direct defence by inducing secondary metabolites (Bruinsma *et al.*, 2007; Van Dam *et al.*, 2004) suggests that the absence of JA accumulation results in higher herbivore abundance and species richness. Indeed, *Nicotiana attenuata* plants that were artificially silenced in a 13-LOX gene harboured higher numbers of herbivores and were even attacked by a species that was never found on control plants in the field (Kessler *et al.*, 2004). This indicates that altering JA accumulation can affect herbivore host selection and herbivore community composition (Kessler *et al.*, 2004; Paschold *et al.*, 2007; Halitschke *et al.*, 2008).

The defence-related gene *TPI*, which encodes a trypsin- and-protease inhibitor, may also play an important role in the observed difference in herbivore community on Rivera and Christmas Drumhead. This gene is a member of the Kunitz trypsin inhibitor family that inhibits proteolytic enzymes within herbivore guts, resulting in reduced insect growth (Schuler *et al.*, 1998; Marchetti *et al.*, 2000). Silencing of *TPI* expression in *A. thaliana* increased *P. rapae* and *B. brassicae* performance in the greenhouse (Broekgaarden, 2008; C Broekgaarden, unpublished data). The higher expression level of *TPI* in Rivera compared to Christmas Drumhead is probably a result of the higher expression level of *LOX2* in Rivera as *TPI* is JA-inducible (Broekgaarden *et al.*, 2007).

The other three defence-related genes that showed higher levels of expression in Rivera compared to Christmas Drumhead may also affect herbivore performance and/or behaviour, resulting in differences in herbivore community composition. Lectins can function as defence proteins against herbivores (Peumans and Van Damme, 1995), Bet v 1 allergen protein is a member of the pathogenesis-related-10 family (Hoffmann-Sommergruber, 2000), and trypsin inhibitors can play a role in plant tolerance to herbivorous insects (Dunaevsky *et al.*, 2005). However, qPCR needs to confirm the microarray results before more detailed studies can be done to determine the role of these genes in shaping herbivore communities.
Intraspecific transcriptional variation in the context of herbivore community composition

From the moment that the plants had been transplanted into the field they were exposed to all kinds of abiotic and biotic stresses such as temperature changes, rainfall, fungi, bacteria, and herbivorous insects that can all induce plant responses and, as a consequence, change gene expression. UV-B radiation, for example, has been shown to increase expression of jasmonate-signalling genes in field-grown *Nicotiana longiflora* (Izaguirre *et al.*, 2003). Early in the season almost no differences in gene expression between the cultivars could be detected (Table 2A), which also holds for the composition of the herbivore community (Fig. 1). Conversely, clear differences in transcriptional profiles as well as in herbivore community composition between Rivera and Christmas Drumhead were observed later in the season (Table 2B; Fig. 1). The putative connection between gene expression and herbivore community composition is schematically shown in Fig. 6 and discussed below.

More than 50% of the genes that showed a higher level of expression in Rivera compared to Christmas Drumhead later in the season had previously been identified as *P. rapae*-responsive in greenhouse experiments (Broekgaarden *et al.*, 2007). Furthermore, it has previously been shown that induction of gene expression upon *P. rapae* feeding lasts for at least three days (Broekgaarden *et al.*, 2007) indicating that all kinds of changes occur in the plant upon herbivore attack beyond a few hours. These previous findings suggest that, besides other environmental factors, herbivore pressure may have a strong influence on shaping a plant’s transcriptional profile in the field and thereby herbivore community composition. Indeed, initial *P. rapae* feeding on two *B. oleracea* cultivars resulted in differential regulation of gene expression upon feeding by sequential herbivores and this resulted in differential effects on performance and abundance of these herbivores on field-grown plants thereby affecting herbivore community composition (Poelman *et al.*, 2008).

Zheng and co-workers (2007) have shown that just a single *P. rapae* larva can induce a fast increase in *LOX2* transcript levels in *B. oleracea*. Our results show that the expression levels of the two defence-related genes *LOX2* and *TPI* were higher in field-grown plants than in greenhouse-grown control plants for both cultivars and comparable to the levels in plants that were challenged for 24 h or 48 h by *P. rapae* under greenhouse conditions. However, the expression levels of *LOX2* and *TPI* were not as high as those after 72 h of feeding by 10 *P. rapae* larvae. This shows that genes are not necessarily expressed to a maximum level, even when more than one *P. rapae* larva is present. The lower gene expression levels in the field may be the result of cross-talk between responses to many different signals. Different herbivores elicit very different transcriptional responses in plants (De Vos *et al.*, 2005) that can have different effects on subsequent herbivores or pathogens.

**Fig. 6.** Schematic model to show the putative connections between the molecular and ecological data obtained later in the season (week 32). Differences in herbivore abundance may be related to differences in gene expression between the two *B. oleracea* cultivars. Based on comparisons of present field data with data from previous greenhouse studies (Broekgaarden *et al.*, 2007, 2008) it is suggested that both cultivars induce the expression of certain defence-related genes (e.g. *LOX2* and *TPI*), but Rivera stronger than Christmas Drumhead.
changes in the plant (Kessler et al., 2004). Thus, the induction of plant responses by herbivory affects subsequent attackers and is mediated by transcription-related changes in the plant (Kessler et al., 2004; Poelman et al., 2008). Unravelling the mechanisms underlying the dynamics of community composition is an exciting process that is now possible through a multidisciplinary approach that connects transcriptomics with metabolomics and community ecology (Kessler and Halitschke, 2007; Bruinsma and Dicke, 2008). To this end, silencing candidate genes, as identified by the microarray analysis, is needed to determine their individual contribution. Additionally, analysing segregating populations based on the two cultivars could lead to the identification of QTLs or expression QTLs (eQTLs) related to ecological communities.

Conclusions

Our results show that clear differences in herbivore community composition between two B. oleracea cultivars develop during the season. These differences could be related to differences in gene expression between the cultivars. While the herbivore populations and gene expression patterns were very similar early in the season, they became different for the two cultivars later in the season. Several defence-related genes showed higher levels of expression in the cultivar that harboured the lowest numbers of herbivores. These data provide an important step towards the analysis of the mechanisms that underlie the dynamics of ecological communities.

Supplementary data

Supplementary data can be found at JXB online.

Supplementary Fig. S1. Photographs of B. oleracea cultivars Rivera and Christmas Drumhead grown in the field.

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References

Adler LS, Schmitt J, Bowers MD. 1995. Genetic variation in defensive chemistry in Plantago lanceolata (Plantaginaceae) and its effect on the specialist herbivore Junonia coenia (Nymphalidae). Oecologia 101, 75–85.

Agrawal AA. 2000. Specificity of induced resistance in wild radish: causes and consequences for two specialist and two generalist caterpillars. Oikos 89, 493–500.

Agrawal AA, Kurashige NS. 2003. A role for isothiocyanates in plant resistance against the specialist herbivore Pieris rapae. Journal of Chemical Ecology 29, 1403–1415.

Ballhorn DJ, Heil M, Pietrowski A, Lieberei R. 2007. Quantitative effects of cyanogenesis on an adapted herbivore. Journal of Chemical Ecology 33, 2195–2208.

Bangert RK, Allan GJ, Turek RJ, Wimp GM, Meneses N, Martinsen GD, Keim P, Whitham TG. 2006. From genes to geography: a genetic similarity rule for arthropod community structure at multiple geographic scales. Molecular Ecology 15, 4214–4228.

Becher M, Talke IN, Kral L, Krämer U. 2004. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator Arabidopsis halleri. The Plant Journal 37, 251–268.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B 57, 289–300.

Bolter CJ, Dicke M, Van Loon JJA, Visser JH, Posthumus MA. 1997. Role of volatiles from herbivore-damaged potato plants in attraction of Colorado potato beetle during herbivory and after its termination. Journal of Chemical Ecology 23, 1003–1023.

Broekgaarden C, Poelman EH, Steenhuis G, Voorrips RE, Dicke M, Vosman B. 2007. Genotypic variation in genome-wide transcription profiles induced by insect feeding: Brassica oleracea–Pieris rapae interactions. BMC Genomics 8, 239.

Broekgaarden C, Poelman EH, Steenhuis G, Voorrips RE, Dicke M, Vosman B. 2008. Responses of Brassica oleracea cultivars to infestation by the aphid Brevicoryne brassicae: an ecological and molecular approach. Plant, Cell and Environment 31, 1592–1605.

Bruinsma M, Dicke M. 2008. Herbivore-induced indirect defense: from induction mechanisms to community ecology. In: Schaller A, ed. Induced plant resistance to herbivory. Berlin, Germany: Springer Verlag, 31–60.

Bruinsma M, Van Dam NM, Van Loon JJA, Dicke M. 2007. Jasmonic acid-induced changes in Brassica oleracea affect oviposition preference of two specialist herbivores. Journal of Chemical Ecology 33, 655–668.

Buckley BA. 2007. Comparative environmental genomics in non-model species: using heterologous hybridization to DNA-based microarrays. Journal of Experimental Biology 209, 1602–1606.

Carroll SB. 2000. Endless forms: the evolution of gene regulation and morphological diversity. Cell 101, 577–580.

Casteel CL, O’Neill BF, Zavala JA, Bilgin DD, Berenbaum MR, DeLucia EH. 2008. Transcriptional profiling reveals elevated CO2 and elevated O3 alter resistance of soybean (Glycine max) to...
Japanese beetles (Popillia japonica). Plant, Cell and Environment 31, 419–434.

Davey MW, Graham NS, Vanholme B, Swennen R, May ST, Keulemans J. 2009. Heterologous oligonucleotide microarrays for transcripomics in a non-model species: a proof-of-concept study of drought stress in Musa. BMC Genomics 10, 496.

De Moraes CM, Mescher MC, Tumlinson JH. 2001. Caterpillar-induced nocturnal plant volatiles repel conspecific females. Nature 410, 577–580.

De Vos M, Van Oosten VR, Van Poecke RMP, et al. 2005. Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Molecular Plant–Microbe Interactions 18, 923–937.

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Van Loon LC, Dicke M, Pieterse CMJ. 2006. Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiology 142, 352–363.

Després L, David J-P, Gallet C. 2007. The evolutionary ecology of insect resistance to plant chemicals. Trends in Ecology and Evolution 22, 298–307.

Dunaevsky YE, Elpidina EN, Vinokurov KS, Belozersky MA. 2005. Protease inhibitors in improvement of plant resistance to pathogens and insects. Molecular Biology 39, 702–708.

Fatouros NE, Broekgaarden C, Bukovinszkie’Kiss G, Van Loon JJA, Mumr M, Huijgens ME, Dicke M, Hilker M. 2008. Male-derived butterfly anti-aphrodisiac mediates induced plant defense. Proceedings of the National Academy of Sciences, USA 105, 10033–10038.

Gao L-L, Klinger JP, Anderson JP, Edwards OR, Singh KB. 2008. Characterization of pea aphid resistance in Medicago truncatula. Plant Physiology 146, 996–1009.

Halitschke R, Kessler D, Kessler A, Baldwin IT. 2008. Shared signals: ‘alarm calls’ from plants increase apparency to herbivores and their enemies in nature. Ecology Letters 11, 24–34.

Heidel AJ, Baldwin IT. 2004. Microarray analysis of salicylic acid and jasmonic acid-signalling in responses of Nicotiana attenuata to attack by insects from multiple feeding guilds. Plant, Cell and Environment 27, 1362–1373.

Hoffmann-Sommergruber K. 2000. Plant allergens and pathogenesis-related proteins. International Archives of Allergy and Immunology 122, 155–166.

Howe GA, Jander G. 2008. Plant immunity to insect herbivores. Annual Review of Plant Biology 59, 41–66.

Hui D, Iqbal J, Lehmann K, Gase K, Saluz HP, Baldwin IT. 2003. Molecular interactions between the specialist herbivore Manduca sexta (Lepidoptera, Sphingidae) and its natural host Nicotiana attenuata. V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. Plant Physiology 131, 1877–1893.

Inbar M, Gerling D. 2008. Plant-mediated interactions between whiteflies, herbivores, and natural enemies. Annual Review of Entomology 53, 431–448.

Izaguirre MM, Scopel AL, Baldwin IT, Ballaré CL. 2003. Convergent responses to stress. Solar ultraviolet-B irradiation and Manduca sexta herbivory elicit overlapping transcriptional responses in field-grown plants of Nicotiana longiflora. Plant Physiology 132, 1755–1767.

Johnson MTJ, Agrawal AA. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (Oenothera biennis). Ecology and Systematics 21, 243–273.

Kaplan I, Denno RF. 2007. Interspecific interactions in phytophagous insects revised: a quantitative assessment of competition theory. Ecology Letters 10, 977–994.

Karban R. 2008. Plant behaviour and communication. Ecology Letters 11, 727–739.

Kazana E, Pope TW, Tibbles L, Bridges M, Pickett JA, Bones AM, Powell G, Rossiter JT. 2007. The cabbage aphid: a walking mustard oil bomb. Proceedings of the Royal Society B: Biological Sciences 274, 2271–2277.

Kessler A, Baldwin IT. 2002. Plant response to insect herbivory: the emerging molecular analysis. Annual Review of Plant Biology 53, 299–328.

Kessler A, Baldwin IT. 2004. Herbivore-induced plant vaccination. Part I. The orchestration of plant defenses in nature and their fitness consequences in the wild tobacco Nicotiana attenuata. The Plant Journal 28, 639–649.

Kessler A, Halitschke R. 2007. Specificity and complexity: the impact of herbivore-induced plant responses on arthropod community structure. Current Opinion in Plant Biology 10, 409–414.

Kessler A, Halitschke R, Baldwin IT. 2004. Silencing the jasmonate cascade: induced plant defenses and insect population. Science 305, 665–668.

Kliebenstein DJ, Kroyman J, Mitchell-Olde T. 2005. The glucosinolate-myrosinase system in an ecological and evolutionary context. Current Opinion in Plant Biology 8, 264–271.

Korth KL. 2003. Profiling the response of plants to herbivorous insects. Genome Biology 4, 221.

Kuśnierzczyk A, Winge P, Midelfart H, Armbruster WS, Rossiter JT, Bones AM. 2007. Transcriptional responses of Arabidopsis thaliana ecotypes with different glucosinolate profiles after attack by polyphagous Myzus persicae and oligophagous Bruchovacryne brassicae. Journal of Experimental Botany 58, 2537–2552.

Lee HS, Wang JL, Tian L, et al. 2004. Sensitivity of 70-mer oligonucleotides and cDNAs for microarray analysis of gene expression in Arabidopsis and its related species. Plant Biotechnology Journal 2, 45–57.

Long JD, Hamilton RS, Mitchell JL. 2007. Asymmetric competition via induced resistance: specialist herbivores indirectly suppress generalist preference and populations. Ecology 88, 1232–1240.

Marchetti S, Delledonne M, Fogher C, Chiaba C, Chiesa F, Savazzini F, Giordano A. 2000. Soybean Kunitz C-II and PI-IV inhibitor genes confer different levels of insect resistance to tobacco (N. tabacum) and potato transgenic plants. Theoretical and Applied Genetics 101, 519–526.

Mendes RS, Evangelista LR, Thomaz SM, Agostinho AA, Gomes LC. 2008. A unified index to measure ecological diversity and species rarity. Ecography 31, 450–456.

Mitchell ND, Richards AJ. 1979. Brassica oleracea L. ssp. oleracea (B. oleracea L.) Miller. Journal of Ecology 67, 1087–1096.
Paschold A, Halitschke R, Baldwin IT. 2007. Coordinated responses: NaCO1 mediates herbivore-induced resistance in Nicotiana attenuata and reveals the role of herbivore movement in avoiding defenses. The Plant Journal 51, 79–97.

Peumans WJ, Van Damme EJ. 1995. Lectins as plant defense proteins. Plant Physiology 109, 347–352.

Pieterse CMJ, Dicke M. 2007. Plant interactions with microbes and insects: from molecular mechanisms to ecology. TRENDS in Plant Science 12, 564–569.

Poelman EH, Broekgaard C, Van Loon JJA, Dicke M. 2008. Early-season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. Molecular Ecology 17, 3352–3365.

Poelman EH, Van Dam NM, Van Loon JJA, Vet LEM, Dicke M. 2009. Chemical diversity in Brassica oleracea affects biodiversity of insect herbivores. Ecology 90, 1863–1877.

Ratzka A, Vogel H, Kliebenstein DJ, Mitchell-Olds T, Kroymann J. 2002. Disarming the mustard oil bomb. Proceedings of the National Academy of Sciences, USA 99, 11223–11228.

Relogio A, Schwager C, Richter A, Ansorge W, Valcarcel J. 2002. Optimization of oligonucleotide-based DNA microarrays. Nucleic Acids Research 30, e51.

Renwick JAA, Radke CD, Sachdev-Gupta K, Städler E. 1992. Leaf surface chemicals stimulating oviposition by Pieris rapae (Lepidoptera: Pieridae) on cabbages. Chemoecology 3, 33–38.

Renwick JAA, Haribal M, Gouinguène S, Städler E. 2006. Isothiocyanates stimulating oviposition by the diamondback moth, Plutella xylostella. Journal of Chemical Ecology 32, 755–766.

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. The Plant Cell 16, 3132–3147.

Riggin-Bucci TM, Gould F. 1996. Effect of surfactants, Bacillus thuringiensis formulations, and plant damage on oviposition by diamondback moth (Lepidoptera: Plutellidae). Journal of Economic Entomology 89, 891–897.

Rishi AS, Nelson ND, Goyal A. 2002. DNA microarrays: gene expression profiling in plants. Reviews in Plant Biochemistry and Biotechnology 1, 81–100.

Root RB. 1973. Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs 43, 95–120.

Schaller F, Schaller A, Stintzi A. 2005. Biosynthesis and metabolism of jasmonates and their role in resistance. Journal of Plant Growth Regulation 23, 179–199.

Schmidt DD, Baldwin IT. 2006. Transcriptional responses of Solanum nigrum to methyl jasmonate and competition: a greenhouse and field study. Functional Ecology 20, 500–508.

Schoonhoven LM, van Loon JJA, Dicke M. 2005. Insect–plant biology, 2nd edn. New York: Oxford University Press.

Schuler TH, Poppa GM, Kerry BR, Denholm I. 1998. Insect-resistant transgenic plants. Trends in Biotechnology 16, 168–175.

Shiojiri K, Takabayashi J, Yano S, Takafuji A. 2002. Oviposition preferences of herbivores are affected by tritrophic interaction webs. Ecology Letters 5, 186–192.

Smith CM, Boyko EV. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata 122, 1–16.

Spoel SH, Koornneef A, Claessens SMC, et. al. 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. The Plant Cell 15, 760–770.

Steppuhn A, Gase K, Krock B, Halitschke R, Baldwin IT. 2004. Nicotine’s defensive function in nature. PLOS Biology 2, 1074–1080.

Thompson GA, Goggin FL. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. Journal of Experimental Botany 57, 755–766.

Traw MB, Dawson TE. 2002. Reduced performance of two specialist herbivores (Lepidoptera: Pieridae, Coleoptera: Chrysomelidae) on new leaves of damaged black mustard plants. Environmental Entomology 31, 714–722.

Van Dam NM, Witjes L, Svatos A. 2004. Interactions between aboveground and belowground induction of glucosinolates in two wild Brassica species. New Phytologist 161, 801–810.

Van Loon JJA, Blaakmeer A, Grieppink FC, Van Beek TA, Schoonhoven LM, De Groot A. 1992. Leaf surface compound from Brassica oleracea (Cruciferae) induces oviposition by Pieris brassicae (Lepidoptera: Pieridae). Chemoecology 3, 39–44.

Voelckel C, Baldwin IT. 2004. Herbivore-induced plant vaccination. Part II. Array-studies reveal the transience of herbivore-specific transcriptional imprints and a distinct imprint from stress combinations. The Plant Journal 38, 650–663.

Walling LL. 2000. The myriad plant responses to herbivores. Journal of Plant Growth Regulation 19, 195–216.

Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, Neumerkel J, Feussner I, Miersch O. 2006. The wound response in tomato: role of jasmonic acid. Journal of Plant Physiology 163, 297–306.

Whitham TG, Bailey JK, Schweitzer JA, et al. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics 7, 510–523.

Wimp GM, Martenssen GD, Floate KD, Bangert RK, Whitham TG. 2005. Plant genetic determinants of arthropod community structure and diversity. Evolution 59, 61–69.

Wittstock U, Agerbirk N, Stauber EJ, Olsen CE, Hippler M, Mitchell-Olds T, Gershenzon J, Vogel H. 2004. Successful herbivore attack due to metabolic diversion of a plant chemical defense. Proceedings of the National Academy of Sciences, USA 101, 4859–4864.

Wu J, Hettenhausen C, Schuman MC, Baldwin IT. 2008. A comparison of two Nicotiana attenuata accessions reveals large differences in signaling induced by oral secretions of the specialist herbivore Manduca sexta. Plant Physiology 146, 927–939.

Zheng S-J, van Dijk JP, Bruinsma M, Dicke M. 2007. Sensitivity and speed of induced defense of cabbage (Brassica oleracea L.): dynamics of BoLOX expression patterns during insect and pathogen attack. Molecular Plant–Microbe Interactions 20, 1332–1345.