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The oriental armyworm (*Mythimna separata*) feeding induces systemic defence responses within and between maize leaves

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Maize (*Zea mays*) is a staple cereal crop cultivated all over the world but that is threatened by various insects. Feeding of the lepidopteran insect *Mythimna separata* triggers defence signalling and increases anti-herbivore benzoxazinoids (Bxs) in the insect-damaged maize leaves. However, the herbivory-elicited within-leaf and leaf-to-leaf systemic signalling in maize remains largely unexplored. Here, we show that simulated *M. separata* herbivory and mechanical wounding elicited increased levels of jasmonic acid (JA), JA–Ile (JA–isoleucine conjugate) and Bxs in the damaged areas and in specific systemic regions within a leaf. Importantly, increased contents of Bxs were detected in a systemic leaf, and consistently, this leaf exhibited increased defence against *M. separata*. Increased JA/JA–Ile and altered transcriptome, including Bx biosynthesis genes, were detected in systemic leaves after wounding or simulated herbivory treatments, although only simulated herbivory induced increase of the contents of Bxs systemically. Promoter and co-expression analysis revealed that transcription factors *bHLH57* and *WRKY34* may regulate Bx biosynthesis genes in systemic leaves. Moreover, leaf ablation experiment indicated that the systemic signal rapidly exited the local leaves within 30 min after elicitation. This study provides new insight into the temporal and spatial regulation of defence responses of maize against lepidopteran insects.

This article is part of the theme issue 'Biotic signalling sheds light on smart pest management'.

1. Background

Plant secondary metabolites provide protection against adverse environmental factors and are required for maximal fitness in nature, and some of the plant secondary metabolites are crucial for plant resistance to herbivores [1]. Biosynthesis of these defensive metabolites is costly, as the resources could otherwise be spent on growth or reproduction. Thus, most defence-related metabolites are inducible, a phenomenon that is thought to be energy- and resource-saving and even herbivore-specific [2–4].

The plant hormones jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA) and ethylene are among the main regulators of inducible defences in plants: the phytohormone-mediated pathways are located in the upstream of the signalling network transforming the damage-associated molecular patterns [5,6] perceived upon wounding or herbivore attack into downstream defence responses, including biosynthesis of defensive metabolites [7–10]. Particularly, JA signalling plays an important role in regulating plant defence against herbivore attack [11–14]. Tomato (*Solanum lycopersicum*), Arabidopsis (*Arabidopsis thaliana*) and the wild tobacco *Nicotiana attenuata* impaired in JA or JA–Ile (JA–isoleucine conjugate)

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biosynthesis are highly susceptible to insects [15–18]. Similarly, genetically modified plants with compromised JA/JA–Ile signalling, such as *N. attenuata* silenced in *COI1* (the receptor in the JA pathway) and Arabidopsis triple mutant *myc2 myc3 myc4* (three basic helix–loop–helix transcription factors (TFs) located the downstream of JA signalling) also exhibit greatly reduced resistance to insects [19,20].

Defences are not only induced in the wounded or insect-attacked local leaves but also in the systemic leaves that are distal from the wounded or insect-damaged sites [21]. Systemic defence is important and necessary for protecting plants by activating the defence before insects relocate to the other parts of the plant [22,23]. Systemic defence was first discovered in tomato: after wounding, systemic leaves were found to have highly elevated activity of proteinase inhibitor I, which has an anti-digestive function in caterpillar midgut [24]. Although the identity of the mobile systemic signal and the mechanism of systemic signal transduction are still largely unknown, studies have indicated that systemic signalling involves reactive oxygen species, electric signals, and is partly JA-dependent [25–27].

Maize is one of the most important cereal crop plants worldwide. Benzoxazinoids (Bxs) are a group of well-studied secondary metabolites that function as defences against herbivores in maize [28]. Some forms of Bxs are stored in the vacuole as glucosides, and upon herbivore attack, they are hydrolysed by glucosidases to release biocidal toxic aglycones [29]. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside (DIMBOA-Glc) is the most prevalent Bx compound in maize seedlings, although its concentration differs among various maize inbred lines [30,31]. A family of three *O*-methyltransferases methylate DIMBOA-Glc into 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc), whose aglycone is toxic to herbivores in wheat and maize [32,33]. The further conversion of HDMBOA-Glc into 6-methoxybenzoxazolin-2(3H)-one (MBOA) confers strong resistance to rice armyworm *Leucania separata* in maize [34]. Moreover, 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3-one glucoside (DIM_2BOA-Glc) and/or 2-hydroxy-4,7,8-trimethoxy-1,4-benzoxazin-3-one glucoside (HDM_2BOA-Glc) provide specific protection against phloem-feeding insects [31].

Maize is able to perceive the fatty acid-amino acid conjugates (FACs) and probably other elicitors, if there are any, in the oral secretions (OS) of the lepidopteran insect [35]. For example, the OS of *Mythimna separata* activate greater levels of JA, SA, ABA and ethylene than those induced by wounding, and more Bxs were also detected in maize treated with *M. separata* OS than in maize treated with wounding [36]. Systemic signalling also occurs in maize infested with insect herbivores. In maize leaves, it was found that wounding induced JA accumulation only at the immediate site of damage, while beet armyworm (*Spodoptera exigua*) crude regurgitant induced JA accumulation in both the local and the distant regions [37]. Similarly, wounding only increased the expression levels of *allene oxide synthase*, TF *MYC7* and *ribosome inactivating protein* at the treatment site, whereas insect elicitor (*N*-linolenoyl-glutamine) strongly induced the expression of these genes throughout the whole leaf [38]. In maize roots, the specialist herbivore *Diabrotica virgifera virgifera* induced hormone-dependent gene expression in maize leaves and primed herbivore resistance in the aboveground tissues [21]. Moreover, *Spodoptera frugiperda* feeding on maize leaves enhanced root resistance to subsequent attack from *D. virgifera* [39].

Yet little is known about whether caterpillar feeding on maize leaves induces defence-related responses in the non-damaged parts of the local leaves and in the neighbouring systemic leaves. In this study, we show that the spatial pattern of Bx accumulation within the treated leaf followed the accumulation of JA/JA–Ile. Leaf ablation experiments further suggest that within 30 min after elicitation, the long-distance systemic signal had been produced and travelled to the systemic leaves where accumulation of JA and JA–Ile was triggered. Increased levels of Bxs were detected in these systemic leaves. Herbivore feeding assays revealed that changes in systemic leaves were translated into elevated resistance, which is most probably Bx-dependent. The response of systemic leaves was further demonstrated via transcriptome analysis, revealing that hundreds of genes were regulated systemically 2 and 6 h after wounding or simulated *M. separata* herbivory. This study sheds light on the spatial regulation of induced resistance to herbivores in maize plants.

2. Results

(a) Jasmonic acid/jasmonic acid–isoleucine conjugate and benzoxazinoid elicitation within a leaf

Maize inbred line A188 has a strong and specific response to *M. separata* OS, including much greater levels of OS-induced JA than those induced by wounding [36]. Given the central role of JA signalling in controlling plant defence responses [40,41], we first investigated the spatial distribution of induced JA/JA–Ile accumulation within a maize A188 leaf. The third leaves (leaf 3) of 18-day-old maize seedlings were evenly allocated into three parts longitudinally, and separated by the midrib, six virtual segments were designated for each leaf 3 (figure 1). Since insect feeding behaviour is very hard to control, these leaf segments were wounded by rolling a pattern wheel, and immediately either 20 µl of water (wounding plus water, as a treatment of mechanical wounding; abbreviated as 'WW' hereafter) or *M. separata* OS (wounding plus OS, as a treatment of simulated herbivory; abbreviated as 'WO' hereafter) were applied to the wounds (illustrated in figure 1). The segments 1, 2 and 3 were treated individually with either WW or WO and JA and JA–Ile were quantified in all leaf segments.

One hour after treating region 1 with WW or WO, we detected strong increases of JA and JA–Ile in region 1 and the adjacent region 2, and to a lesser extent in region 3, with no significant differences between WW and WO (figure 1*a*); by contrast, there were no changes in the levels of these hormones in the other areas of the leaf, including region 4, which was adjacent to region 1 but separated by the midrib (figure 1*a*). When region 2 was treated, we found increases of JA and JA–Ile in the treated area and in region 3, but not in region 1 (figure 1*b*). Again, no increases in hormone concentrations were detected in regions 4–6 on the other side of the midrib, suggesting that the midrib may act as a barrier for the inducing signal. After region 3 was treated, JA and JA–Ile levels not only highly increased in the treated area but also slightly elevated in region 6 on the other side of the midrib, and in the treated region 3, WO induced 59 and 29% greater levels of JA and JA–Ile, respectively, than did WW (figure 1*c*). Thus, it is very likely that wounding- and the OS-induced systemic signal travel

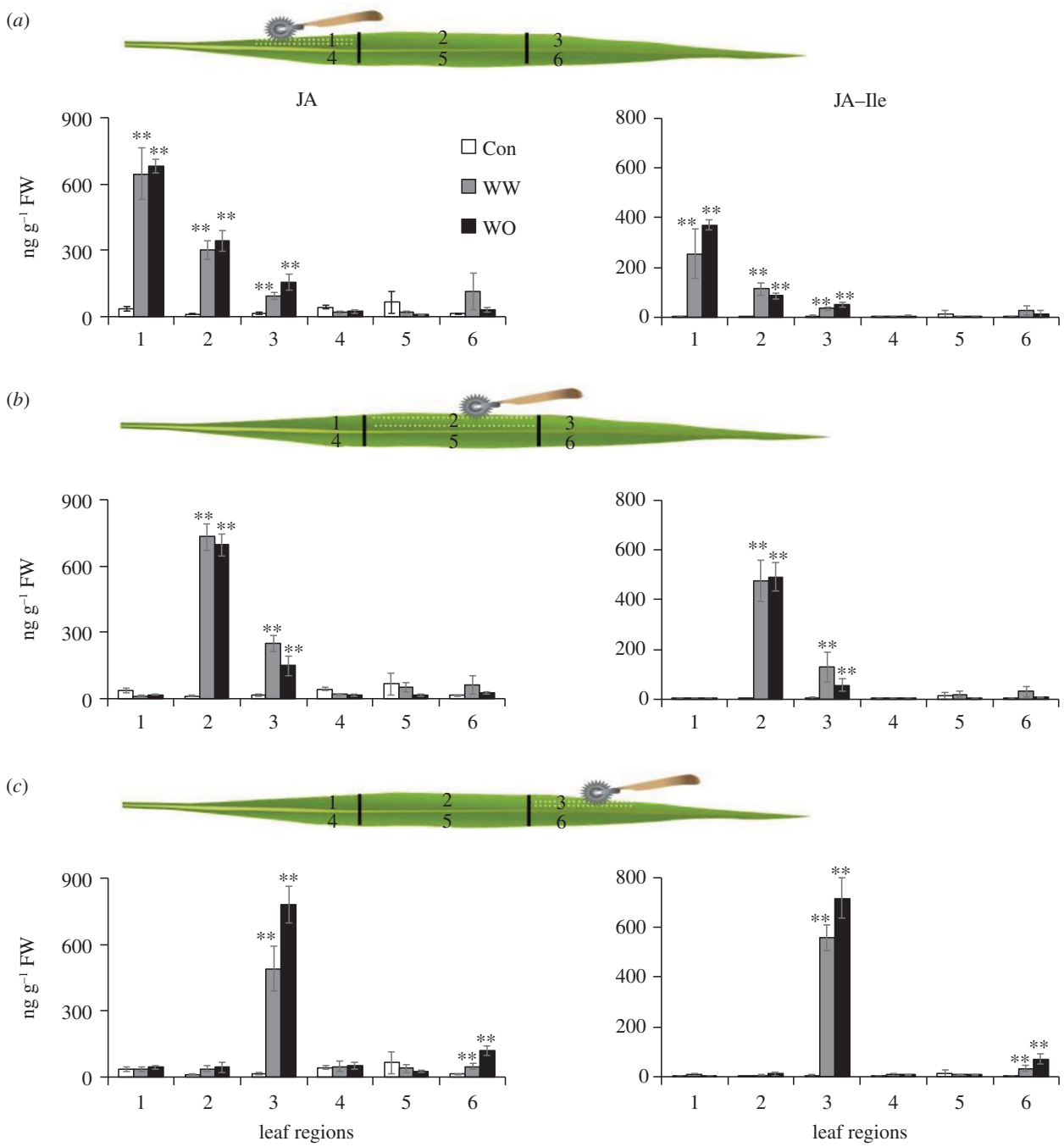

Figure 1. Spatial distribution of JA and JA–Ile within the treated leaf. The third maize leaves were treated with WO or WW at region 1 (*a*), 2 (*b*) or 3 (*c*), as illustrated by the white dotted lines in the schematics. The six sections of the leaves were harvested 1 h after the treatments and the same sections of untreated leaves served as controls (Con). The levels of JA (left column) and JA–Ile (right column) were quantified for all sections. Asterisks indicate significant differences between induced and control samples of the same leaf region (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$). Data are mean ± s.e. ($n = 5$). FW, fresh weight.

from the leaf base to the tip but not from tip to base and the signal can hardly be translocated to the other side of the leaf over the midrib. Furthermore, this signal can induce the accumulation of phytohormones JA and JA–Ile.

Maize responds to herbivore attack by strongly increasing the contents of defence-related Bxs, including DIM_2BOA-Glc, HDMBOA-Glc, MBOA and HDM_2BOA-Glc [33,34,42–44]. To analyse the pattern of Bx accumulation within the treated leaf, leaf segments 1, 2 and 3 were individually treated with WW or WO, and the Bx levels were quantified in all regions 2 days after the treatment. WO or WW treatment in region 1 led to a substantial local increase of all quantified Bxs and WO tended to induce greater levels of Bxs than did WW treatment (figure 2*a*). Compared with WW treatment, WO treatment in region 1 increased greater levels of Bxs in region 2. No responses of Bxs were detected in the other areas of the leaf (regions 3–6) (figure 2*a*). When region 2 was treated with WW, only the concentrations of HDM_2BOA-Glc, but not the other Bxs detected, increased locally and in region 3, whereas after WO treatment all four Bxs accumulated highly in regions 2 and 3 but not in region 1 (figure 2*b*). Again, no increase in Bx concentrations was detected in regions 4–6 on the other side of the midrib. After WO elicitation in region 3, we found highly increased contents of DIM_2BOA-Glc, MBOA, HDMBOA-Glc and HDM_2BOA-Glc exclusively in region 3, while there were no changes of these Bxs in the systemic tissues; only HDM_2BOA-Glc responded locally to WW treatment in

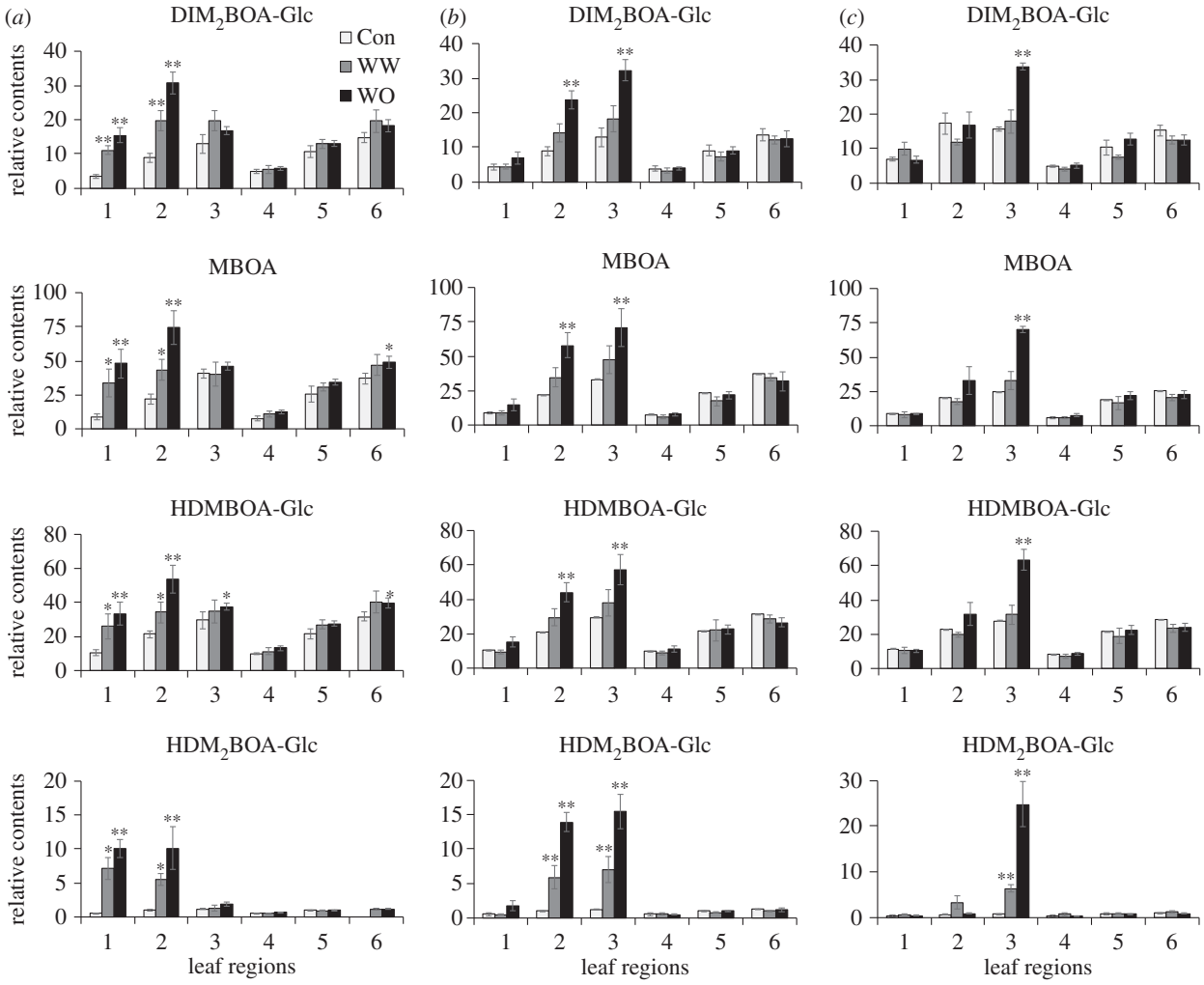

Figure 2. Spatial distributions of Bx metabolites within the treated leaf. The third maize leaves were treated with WO or WW at region 1 (*a*), 2 (*b*) or 3 (*c*), as illustrated by the white dotted lines in the schematics in figure 1. The six sections of the leaves were harvested 48 h after the treatments and the same sections of untreated leaves served as controls (Con). The levels of different Bxs were quantified for all sections. Asterisks indicate significant differences between induced and control samples of the same leaf region (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$). Data are mean ± s.e. ($n = 5$).

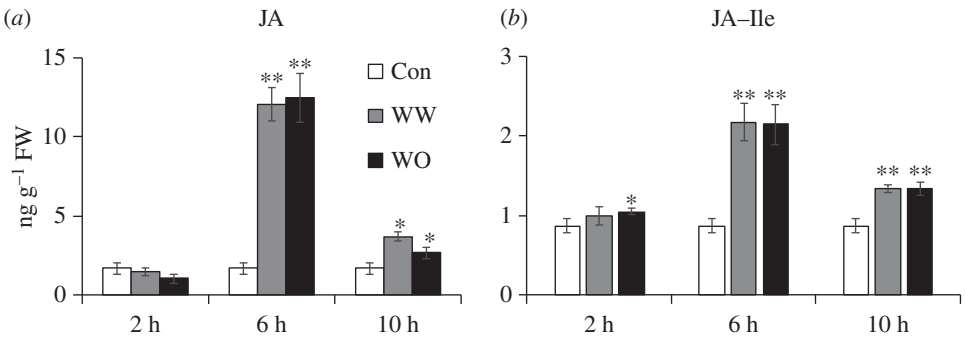

Figure 3. Systemic JA and JA–Ile levels after WO and WW treatment. The third maize leaves were elicited with WW and WO at the indicated times prior to harvest. The systemic leaves (fourth leaves) of the treated and untreated control plants (Con) were harvested to determine the levels of (*a*) JA and (*b*) JA–Ile. Asterisks represent significant differences between treated and control samples (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$). Data are mean ± s.e. ($n = 7$). FW, fresh weight.

region 3 (figure 2*c*). Thus, wounding- and OS-induced Bx responses are strictly from base to tip and the midrib seems to act as a barrier for Bxs or the induced systemic signal.

(b) Wounding- and simulated herbivory-induced defence responses in systemic leaves

To examine whether leaf herbivory induces jasmonate responses in the systemic leaves, the levels of JA and JA–Ile in systemic leaves were determined. Two hours after WW or WO treatment on leaf 3, no changes of JA or JA–Ile levels were detected in leaf 4. However, JA levels increased 6- and 1-fold at 6 h and 10 h, respectively, after WW or WO treatment (figure 3*a*). Similarly, JA–Ile showed about onefold induction only at 6 h after WW or WO treatment (figure 3*b*). Next, we profiled the Bx compositions in the systemic leaf (leaf 4) 48 h after WW and WO treatment on leaf 3 (figure 4*a*). In the systemic leaf 4, DIM_2BOA-Glc, MBOA,

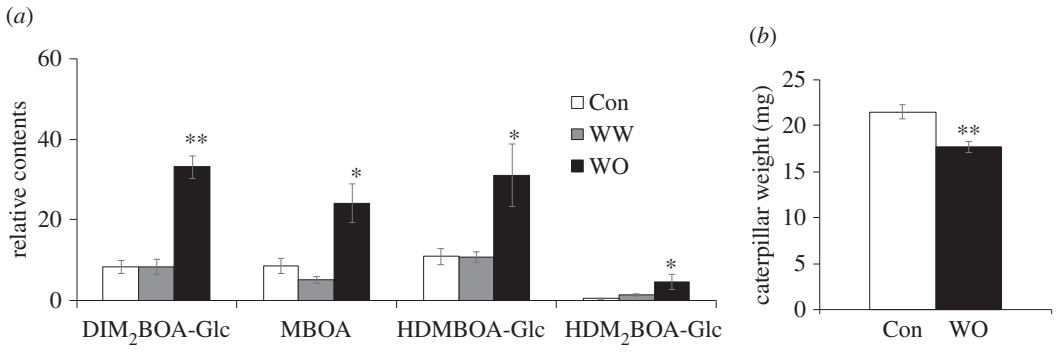

Figure 4. Herbivore defence-related traits in systemic leaves. The third leaves of maize seedlings were elicited with WW or WO, and untreated maize seedlings served as controls (Con). (*a*) Bx levels in systemic (fourth) leaves 48 h after treatment (mean ± s.e.; $n = 7$). (*b*) *M. separata* masses (mean ± s.e.; $n = 30$) after feeding for 24 h on the systemic (fourth) leaves of WO-pretreated and control maize seedlings. Asterisks represent significant differences between treated and control samples (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$).

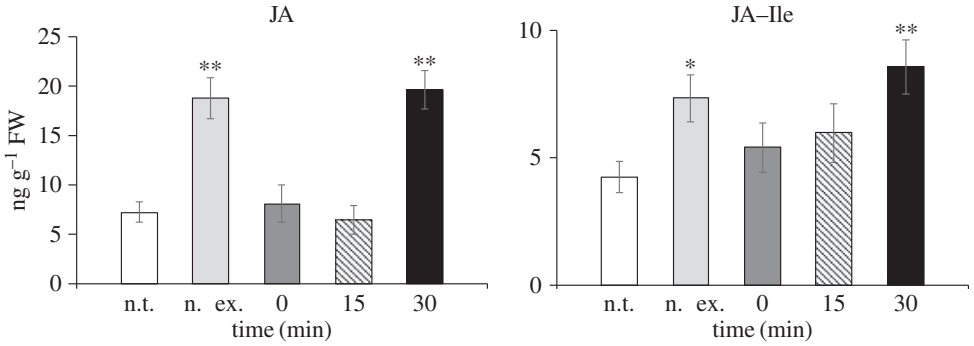

Figure 5. Systemic JA and JA–Ile levels after treatment and leaf excision. Local leaves (third leaves) were elicited with WO, and the treated leaves were excised at the indicated times after treatment. The systemic (fourth leaves) JA and JA–Ile levels were determined 6 h after elicitation. The fourth leaves of plants, whose third leaves were treated with WO but were not excised, were harvested at 6 h (named 'n. ex.' group). The fourth leaves of control plants, whose third leaves were untreated, were also harvested at 6 h (named 'n.t.' group). Asterisks represent significant differences between treated and control group (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$). Data are mean ± s.e. ($n = 9$). FW, fresh weight.

HDMBOA-Glc and HDM_2BOA-Glc were specifically elicited by WO, but their contents were unchanged after WW treatment in systemic leaves (figure 4*a*).

To further investigate the defensive effect of the systemically induced responses on plant resistance to insects, maize plants were pretreated with WO on leaf 3, and 48 h later, *M. separata* larvae were allowed to feed on leaf 4 for 24 h. Compared to those in the control group (not pretreated), caterpillars on pretreated plants gained 22% less average mass (figure 4*b*), suggesting that the systemic changes of Bxs and probably other defensive metabolites conferred resistance to the subsequently infested caterpillars.

(c) Determination of the speed of systemic signal

To examine the time required for the exit of systemic signal from wounded leaf to systemic leaves, the third leaves were treated with WO and then were excised from the base at 0, 15 and 30 min, and the systemic undamaged fourth leaves were harvested 6 h after the initial WO treatment. In addition, in another group of maize plants (positive controls), the third leaves were similarly treated but without being excised and the fourth leaves were harvested at 6 h. The JA and JA–Ile contents in these systemic leaves were quantified.

It was found that excision 30 min after treating the third leaves with WO still resulted in systemic accumulation of JA and JA–Ile to the levels that were comparable with those in the positive controls (figure 5). However, excision of the damaged leaves 0 and 15 min after WO treatment resulted in no change in systemic JA and JA–Ile, as in the untreated controls (figure 5). These results demonstrate that systemic production of JA and JA–Ile involves a signal that exits the elicited leaf within 15–30 min after tissue damage.

(d) Transcriptomic response to mechanical wounding and simulated herbivory in systemic leaves

To investigate the global transcriptomic changes in systemic leaves, the third leaves of maize seedlings were elicited with WW or WO, and the forth leaves were collected after 2 and 6 h and analysed with RNA-seq. In total, approximately 40 000 transcripts were detected (electronic supplementary material, table S1), and among these, 1088 transcripts were at least onefold upregulated or 50% downregulated in the systemic leaves (electronic supplementary material, table S2). At 2 h, WO treatment induced many more transcriptional changes (276 differentially expressed genes (DEGs)) than did WW (70 DEGs) with only 31 commonly regulated genes; 6 h after WO and WW treatment, the systemic leaves exhibited 349 and 393 DEGs, respectively, and 191 genes were commonly regulated by both treatments. There were 15 genes commonly upregulated 2 and 6 h after WW and WO

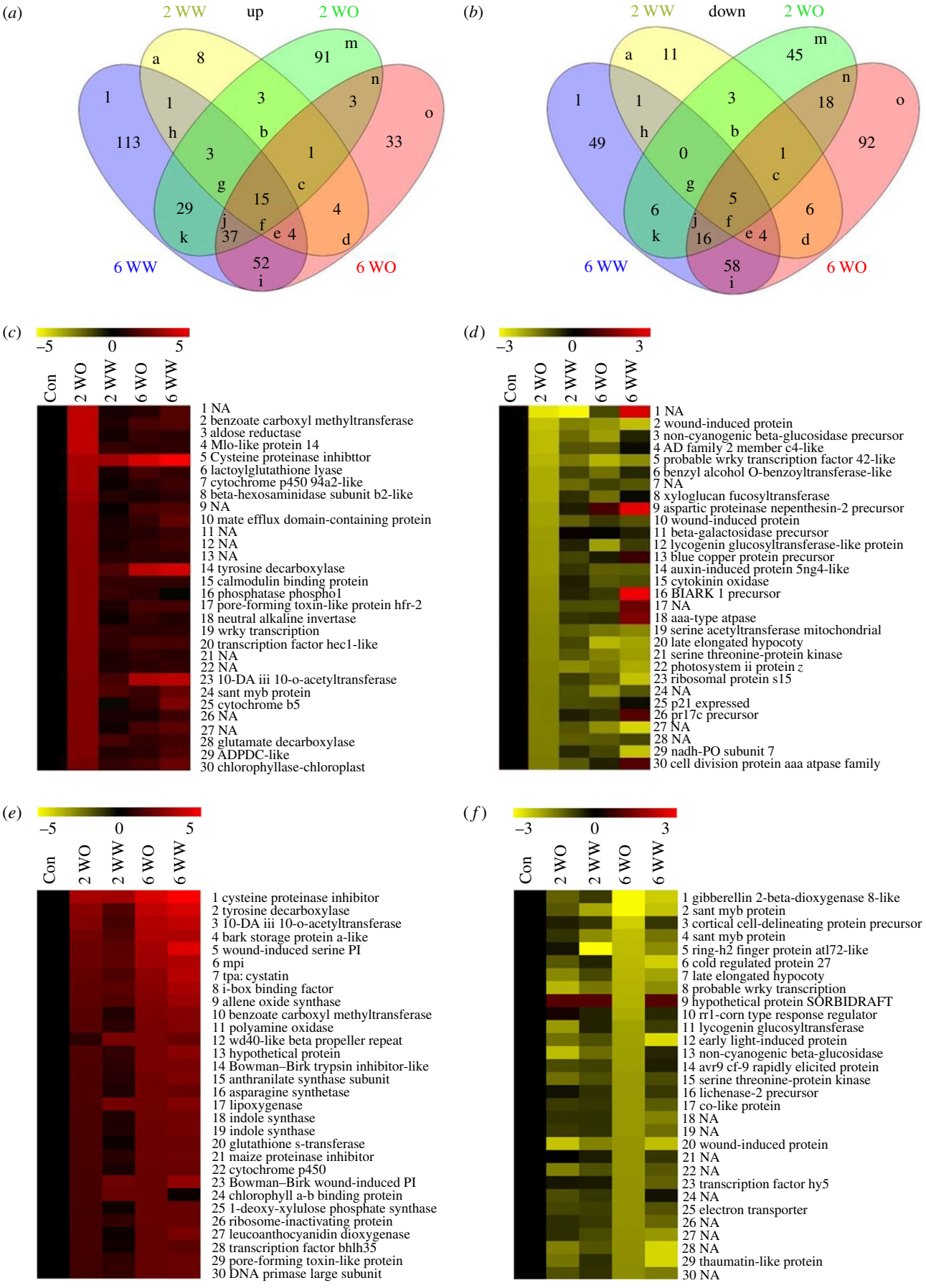

Figure 6. Expression profiles of upregulated and downregulated maize systemic transcripts. Maize leaves (third leaves) were treated with WW or WO, and systemic (fourth leaves) samples were collected at 2 and 6 h. The fourth leaves of untreated maize seedlings were harvested as controls (Con). (*a*,*b*) Venn diagrams depict the numbers of (*a*) upregulated and (*b*) downregulated transcripts. Details of the genes in different areas (denoted by the small letters) of the Venn diagrams can be found in electronic supplementary material, tables S3 and S4. (*c*,*d*) Heatmaps of the relative gene expression levels of the 30 most upregulated (*c*) and downregulated (*d*) genes 2 h after WO treatment. (*e*,*f*) Heatmaps of the relative gene expression levels of the 30 most upregulated (*e*) and downregulated (*f*) genes 6 h after WO treatment. The number 2 or 6 before WW or WO indicates the time (h) of sample harvested after WW or WO treatment. NA, not annotated.

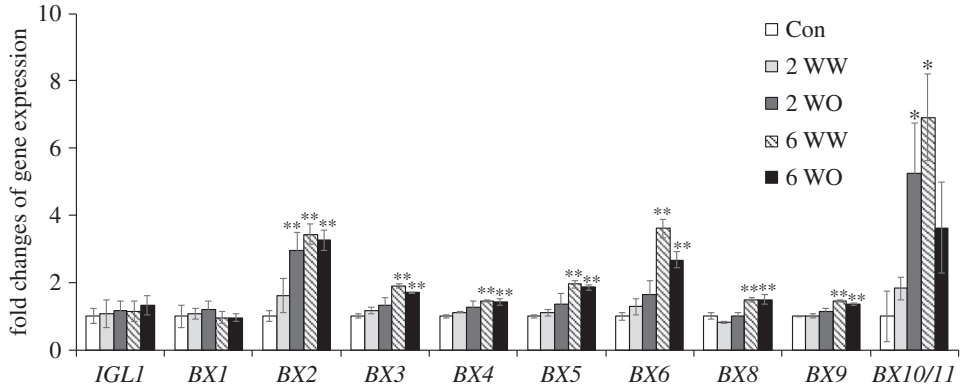

Figure 7. Transcriptional changes of *BX* genes in systemic leaves. Maize leaves (third leaves) were treated with WW or WO 2 and 6 h before the systemic leaf samples (fourth leaves) were collected. The fourth leaves from untreated plants served as controls (Con). Asterisks represent significant differences between treated and control group (Student's *t*-test; *, $p < 0.05$; **, $p < 0.01$). Data are mean $\pm$ s.e. ($n = 3$). The number 2 or 6 before WW and WO indicates the time (*h*) of sample harvested.

treatment, and these included *cysteine proteinase inhibitor* (GRMZM2G312061), *lipoxygenase* (GRMZM2G156861) and *maize proteinase inhibitor* (GRMZM2G042789), which are known to be related to defence against insects [45,46] (figure 6*a*; electronic supplementary material, table S3). Similarly, only 5 genes were commonly downregulated in all samples, including a *sant myb protein* (GRMZM2G117497) and a *wound-induced protein* (GRMZM2G106393) (figure 6*b*). Thus, the transcriptomic regulation of systemic leaves seems to be very dynamic and perception of OS leads to specific responses.

The top 30 most upregulated genes identified in samples harvested 2 h after simulated herbivory included *benzoate carboxyl methyltransferase* (GRMZM2G063438), *cysteine proteinase inhibitor* (GRMZM2G312061) and *cytochrome p450 94a2-like* (GRMZM2G177668) (figure 6*c*; electronic supplementary material, table S3). Most of these highly induced genes' transcript levels were lower in response to WW treatment at 2 h and exhibited even decreased levels after 6 h (figure 6*c*; electronic supplementary material, table S3). Six hours after WW and WO treatment, the highly induced genes included *wound-induced serine proteinase inhibitor* (*PI*) (GRMZM2G156632), and *maize proteinase inhibitor* (GRMZM2G028393) (electronic supplementary material, table S3). We further analysed transcripts, which were downregulated at least 50% 2 h after WO treatment in the systemic leaves (electronic supplementary material, table S4). These downregulated genes included *wound-induced protein* (GRMZM2G106393) and *benzyl alcohol O-benzoyltransferase-like* (GRMZM2G122503) (figure 6*d*). Compared with our WO treatment, WW did not suppress the transcript levels of these genes so strongly. *Cysteine proteinase inhibitor* (GRMZM2G312061), *tyrosine decarboxylase* (GRMZM2G108514) and *10-deacetylbaccatin iii (10-DA iii) 10-o-acetyltransferase* (GRMZM2G079616) were among the 30 most upregulated transcripts found in samples harvested 6 h after WO treatment in systemic leaves. Unlike those in the samples of 2 h treatments, many of these highly induced transcripts exhibited similar levels between WW and WO treatment at 6 h, and their levels were generally higher than their respective levels at 2 h (figure 6*e*). The top 30 most downregulated transcripts in the systemic leaf harvested 6 h after WO treatment included *gibberellin 2-beta-dioxygenase 8-like* (GRMZM2G006964), *sant myb protein* (GRMZM2G117497) and *cortical cell-delineating protein precursor* (GRMZM2G162276) (figure 6*f*). Notably, the expression levels of these most downregulated transcripts were higher in the systemic leaf harvested 2 h after WW or WO treatment and 6 h after WW treatment. These data suggest that the elicitors in the OS [36] play an important role in modifying the transcriptomic responses to wounding and different amounts of OS may give different quantitative results. Furthermore, similar to what was found among the highly regulated genes in the local maize leaves after wounding or simulated herbivory [36], many regulated genes in the systemic leaves were not annotated, suggesting that maize regulates various unique genes when challenged by *M. separata*.

Specifically, we inspected the expression profiles of *BX* genes, which encode enzymes important for the biosynthesis of Bxs [47,48]. Owing to their low abundance, expression values of *BX13* and *BX14* could not be obtained from RNA-seq data. In the systemic leaves, *IGL1* and *BX1*, which encode the first two enzymes in the Bx biosynthesis, did not show changes under all conditions. At 2 h, WW treatment hardly induced any Bx transcript accumulation, while increased levels of *BX2* and *BX10/11* (*BX10* and *BX11* are two homologous genes [30] whose transcripts could not be distinguished in the RNA-seq data) were detected in the samples treated with WO (figure 7). Intriguingly, *BX2*, *BX3*, *BX4*, *BX5*, *BX6* and *BX10/11* were induced 6 h after both WW and WO treatments, and the expression levels of *BX6* and *BX10/11* were even lower in the WO-treated systemic leaves than in the WW-treated ones (figure 7). Thus, in systemic leaves, the expression of Bx biosynthesis genes was not correlated with the findings that WW treatment did not change systemic Bx contents, while WO treatment did.

(e) Systemically regulated transcription factors

Differentially regulated TFs were determined by searching the Plant Transcription Factor Database (http://planttfdb.cbi.pku.edu.cn). We identified 51 unique TFs from 15 different families that were up- or downregulated at least by one treatment in the systemic leaves (electronic supplementary material, tables S5 and S6). The most abundant TF families were bHLH (10 DEGs), MYB-related (7 DEGs), WRKY (8 DEGs) and the MYB family (8 DEGs) (electronic supplementary material, table S5). In general, the number of regulated TFs was congruent with the divergent pattern of gene expression between WW and WO. Two hours after elicitation, 1 and 8 TFs were specifically upregulated by WW

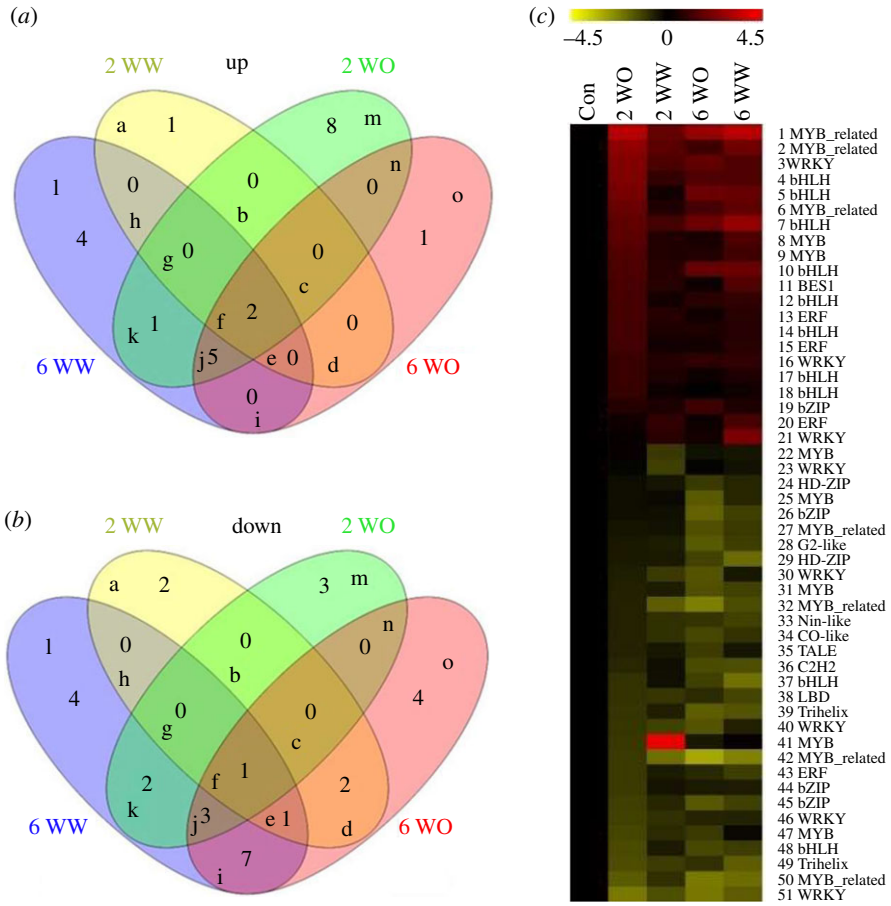

Figure 8. Expression profiles of upregulated and downregulated maize TFs in systemic leaves. Maize leaves (third leaves) were treated with WW or WO, and systemic (fourth leaves) samples were collected at 2 and 6 h. The fourth leaves of untreated maize seedlings were harvested as controls (Con). (*a*,*b*) Venn diagrams depict the numbers of upregulated (*a*) and downregulated (*b*) TF genes. Details of the genes in different areas (denoted by the small letters) of the Venn diagrams can be found in electronic supplementary material, tables S5 and S6. (*c*) Heatmap of the relative gene expression levels of all the regulated TF genes. The number 2 or 6 before WW or WO indicates the time (h) of sample harvested after WW or WO treatment. The details of genes in (*c*) can be found in electronic supplementary material, table S5.

and WO, respectively; 4 and 1 TF were specifically upregulated at 6 h by WW and WO (figure 8*a*; electronic supplementary material, table S5). We found that 2 TFs were upregulated in all samples (figure 8*a*; electronic supplementary material, table S5). Among the downregulated TFs, 2 and 3 were specific for samples collected 2 h after WW and WO treatment, respectively (figure 8*b*; electronic supplementary material, table S6). Four TFs were specific for 6 h WW-treated samples, and the same number of TFs were specially downregulated in 6 h WO-treated ones (figure 8*b*; electronic supplementary material, table S6). Only one TF was commonly downregulated in all samples (figure 8*b*; electronic supplementary material, table S6). A heat map was used to directly visualize the changes in expression levels of these TFs (figure 8*c*). Twenty-one and 30 TFs were upregulated and downregulated under at least one of the treatment conditions, respectively. It is possible that changes in the gene expression of these TFs may drive the transcriptional and thereby also metabolic responses to insect herbivory in the systemic maize leaves.

We hypothesized that the Bx biosynthesis genes, which were induced 2 and 6 h after WW and WO, are regulated by TFs in systemic leaves. To further examine whether these 51 TFs could bind to the promoters of Bx biosynthesis genes in silico, we used the Plant Transcription Factor Database [49] to identify the transcription factor binding sites (TFBS) in the 2 kb upstream of start codons (the putative promoter regions) of *BX2*, *BX6* and *BX10/11*, as these genes showed relatively highly elevated transcript levels after local WW and/or WO treatment. Among the systemically regulated TFs, *bHLH57* (GRMZM2G159937) was predicted to bind *BX2* promoter (electronic supplementary material, table S6), and it was found that the regulation of *bHLH57* and *BX2* were similar: *bHLH57* was induced 2.4- to 6.5-fold and *BX2* was induced 3.0- to 10.7-fold 2 or 6 h after WW or WO treatment (electronic supplementary material, table S6). Another TF, *WRKY34* (GRMZM2G057116), was predicted to bind the promoters of *BX6*, *BX10* and *BX11* (electronic supplementary material, table S6). Since the level of *WRKY34* was not altered at 2 h by either WW or WO but was elevated 4.6-fold 6 h after WW treatment, we speculated that *WRKY34* may regulate *BX6* and *BX10/11* in later time point (6 h).

To gain insight into the possible regulation of these TFs, the transcript levels of phytohormone biosynthesis-related genes in systemic leaves were examined, but we did not find obvious patterns of regulation in these genes (electronic supplementary material, table S7), suggesting that phytohormone-independent pathways may be involved in controlling the expression levels of these TFs and the production of BXs in systemic leaves.

(f) Comparison of local and systemic transcriptomic responses

To gain more insight into the common and specific responses in local and systemic leaves, we compared the elicited transcriptomic changes between local and systemic leaves using our previously published local transcriptomic data [36] and the transcriptomic data from systemic leaves obtained in this study (electronic supplementary material, table S8). Six hours after WO treatment, there were 116 common DEGs in both local and systemic leaves, and these commonly regulated genes were 0.93 and 33.2% of local and systemic overall DEGs, respectively (electronic supplementary material, figure S1). Similarly, 6 h after WW treatment, 167 DEGs were found to be common between local and systemic leaves, and these common DEGs were 1.85 and 42.5% of local and systemic overall DEGs (electronic supplementary material, figure S1). One hundred thirty-two and 106 genes were specifically regulated in systemic leaves 6 h after WO and WW treatments, respectively; by contrast, many more DEGs (5708 and 2235) were specifically found in local leaves 6 h after WO and WW (electronic supplementary material, tables S8 and S9). Thus, WW and WO induce much starker changes in local leaves than in systemic ones, and a big portion of the systemic DEGs are in common with those in local leaves.

3. Discussion

Herbivory-induced defence responses have been studied in insect-infested or simulated herbivory-treated maize leaves [36,50,51], but little is known about how maize responds to these stresses spatially. This study attempted to characterize maize response to wounding and simulated *M. separata* herbivory within a leaf and between leaves by analysing RNA-seq data, Bx contents, and phytohormones levels, which are important anti-herbivore traits.

We found that within a leaf (maize cv. A188 in this study), JA and JA–Ile were elicited at the wounded sites and in specific systemic regions after both WW and WO treatment. By contrast, Engelberth *et al*. [37] treated the different regions of maize (cv. Kandy King and Bonus) leaves with volicitin (a hydroxylated FAC), wounding and crude regurgitant (CRE) from beet armyworm *Spodoptera exigua*; increases in JA and 12-OPDA levels were only observed in the treated regions after wounding, whereas application of CRE and volicitin induced JA in distal regions of treated leaves. Furthermore, when the middle region of a maize (cv. B73) leaf was treated with *Spodoptera littoralis* feeding for 24 h, no alteration of JA and Bx content was detected in systemic regions within the treated leaf [50]. The discrepancy between these previous studies and ours suggests that different maize genotypes may have distinct spatio-temporal organization of systemic responses within-leaf to wounding and insect feeding.

Within the treated leaf, the systemic signal seems to travel from the base to tip in a unidirectional manner. Namely only the systemic regions close to the tip showed induction of JA/JA–Ile or Bxs. However, responses were also detected in systemic leaves, suggesting that the systemic signal does not travel solely from the base to the tip in the damaged leaves but also moves in the opposite direction to the distal leaves. Similarly, in the wild tobacco *N. attenuata*, treating different regions of a leaf with simulated insect feeding also indicated that the movement of the systemic signal is from the base to tip [52], but the expression of *trypsin proteinase inhibitor*, a gene encoding a precursor of anti-digestive peptides [3], was detected in almost all systemic leaves as well [52,53]. It is possible that the systemic signal travels from the base to the tip through the lamina of the leaf, and during this process, it enters the midvein; thereafter, through the midvein, the systemic signal is transported from tip to base and then to the systemic leaves, activating defence-related responses. It is noteworthy that treating regions 1 and 2 did not induce JA in the systemic regions of the other side of midvein (regions 4, 5 and 6); by contrast, after region 3 was treated with WW or WO, the systemic signal travelled across the midvein to region 6 and induced JA accumulation (figure 1). It is possible that the midvein functions as a barrier, preventing the systemic signal from moving across; however, when the tip region (region 3) is treated, the adjacent midvein, which is smaller than the midvein at the base part, allows a small amount of systemic signal to enter the other side of the leaf. A similar phenomenon was also detected in *N. attenuata*: The simulated herbivory-induced systemic signal could not move across the midvein at the base side of a leaf, but could be translocated across the midvein in the tip region [52]. Moreover, within a maize leaf, the levels of JA and JA–Ile were higher in the damaged leaf regions than in undamaged leaf regions (figure 1); however, the levels of BXs in undamaged leaf regions (near damaged regions) were higher than those in damaged regions (figure 2). These data suggest that in addition to JA, other signalling pathways are also involved in the regulation of BX accumulation.

As indicated by our bioassay, elevated contents of Bxs and probably other defensive metabolites in systemic leaves conferred resistance to maize against insects that may arrive later. Thus, understanding the mechanism by which maize regulates systemic accumulation of Bxs is particularly important. In systemic leaves, Bxs were elevated only after WO treatment, but not after WW (figure 4*a*), indicating that WO- and WW-induced systemic signals are likely to be qualitatively different. RNA-seq analysis also indicated that WW and WO treatment led to largely distinct transcriptomic changes in systemic leaves (figure 6*a*,*b*). Probably, WW and WO could induce the same systemic signal with different intensities (stronger after WO), but it is also possible that these treatments activate different but yet overlapping multiple systemic signals. Corroborating this scenario, recently, an Arabidopsis–dodder (*Cuscuta australis*)–tobacco system was used to dissect the nature of the systemic signal, and it was found that systemic signalling likely involves multiple mobile signals and the signals are controlled by JA-dependent and -independent pathways [54]. Given the largely overlapping transcriptomic responses in local and systemic leaves after WW and WO, the mobile systemic signal(s) may also operate in the local leaves, before moving to the systemic leaves, and in the systemic leaves after being translocated therein.

Applying JA to maize induced accumulation of Bxs, suggesting that JA regulates Bx biosynthesis [33]. In local leaves, WW and WO treatment induced large differences in JA levels (around onefold differences), but these treatments did not lead to big differences in Bx contents [36]. After WO and WW treatment, we detected similar levels of slightly

increased JA or JA–Ile in the systemic leaves; furthermore, WW and WO induced similar expression of Bx biosynthesis genes (WO-induced *BX6* and *BX10/11* levels were even lower than those induced by WW) in systemic leaves, but Bxs increased strongly only after WO treatment in systemic leaves, but not after WW treatment. Thus, it is likely that in maize systemic leaves JA may not be crucial for Bx accumulation and probably gene expression and metabolite accumulation do not align. Moreover, accumulation of Bxs cannot always be predicted from expression pattern of biosynthetic genes. Furthermore, transcript levels of phytohormone biosynthesis-related genes in systemic maize leaves were not differentially regulated after WW and WO treatments (electronic supplementary material, table S6), suggesting that non-phytohormone-related signalling networks may be involved in modulating BXs in systemic leaves. Simulated *M. separata* feeding induced much lower numbers of DEGs in the systemic leaves than in the local leaves; nevertheless, Bx biosynthetic genes are still similarly induced in local and systemic leaves. Taking advantage of the relatively small number of DEGs of TFs in systemic leaves, via an in-silico analysis we predicted that *bHLH57* (for *BX2*) and *WRKY34* (for *BX6* and *BX10/11*) may control the expression of Bx biosynthesis genes. Biochemical and genetic analyses are needed to confirm whether these TFs really function as regulators of Bx biosynthetic genes.

In *N. attenuata*, leaf ablation experiment indicated that the systemic signal exits the local leaf within 15 min [53]. In Arabidopsis, wounding a leaf resulted in systemic accumulation of JA–Ile within 5 min [55], and another study revealed that the speed of the mobile signal triggering systemic jasmonate synthesis was in the range 3.4–4.5 cm min^{-1} [56]. These measurements from dicotyledonous plants suggest species-specific speed of mobile signal translocation. In maize, our measurements revealed that the systemic signal travels slightly slower than in *N. attenuata* or Arabidopsis, as 15–30 min was needed for it to exit the treated leaves. Whether systemic signals are generally slower in monocots than in eudicots requires many more comparisons between various species, and the factors influencing the speed of systemic signals remain to be studied.

4. Material and methods

(a) Plant growth and oral secretions collection

Maize (*Zea mays* cv. A188) seeds were germinated directly in 1 L pots filled with commercial potting soil (Pindstrup Blond Gold, http://www.pindstrup.com) and grown under natural light conditions (about 12–14 h day length) in the greenhouse (25 ± 4°C day, 20 ± 4°C night). The plants used for all experiments were approximately 18 days old, when the fourth leaves were fully expanded from the whorl. Eggs of *Mythimna separata* were purchased from the Generalpest Company (www.genralpest.com). For the collection of *M. separata* OS, larvae were reared on maize until the third to fifth instar. To provoke regurgitation, caterpillars were gently squeezed using stork bill forceps and OS were collected with a pipette and kept on ice; OS were aliquoted before being stored at −80°C.

(b) Plant treatments

To measure responses in systemic leaves, the third leaves (local) of maize were wounded with a pattern wheel, and 20 µl of water or *M. separata* OS were gently rubbed into the puncture wounds (WW and WO treatment, respectively). Local and/or systemic leaves (the fourth leaves) were harvested at indicated times after treatments, and the leaves from intact plants were sampled as controls. Leaf samples were snap-frozen in liquid nitrogen and stored at −80°C until further use. All experiments, except for the RNA-seq analysis, were repeated twice or three times to ensure data reproducibility. The number of replicates for each experiment varied and is indicated in the respective figure legends. To measure the hormone and Bx distribution within-leaves, the third leaf was virtually divided into six regions, and regions 1, 2 or 3 were individually induced with WW or WO treatments. Samples of all regions were harvested 1 or 48 h after treatments for hormone or Bx analysis, respectively.

To investigate the speed of signal transduction between leaves, the third leaf was induced with WO and removed (by excising from base with a scalpel) at 0, 15, 30 and 60 min after the treatment; untreated plants, and plants induced with WO whose local leaves were retained, were used for comparisons. Six hours after the initial WO treatment, systemic leaf samples were harvested for hormone analysis.

(c) Herbivore assays

Mythimna separata neonates were used for the caterpillar growth assay. Following a 48 h WO pretreatment at leaf 3, *M. separata* neonates were infested onto the leaf 4, and their masses were recorded after 24 h of feeding.

(d) Phytohormone profiling

JA and JA–Ile were quantified according to a method described previously [52]. In brief, 150 mg of frozen leaf powder was extracted with ice-cold ethyl acetate spiked with 20 ng of D_6-JA and 5 ng of $^{13}C_6$-JA–Ile. After centrifugation at 13 000 *g* for 10 min at 25°C, supernatants were transferred to fresh 2 ml Eppendorf tubes. Each pellet was re-extracted with 0.5 ml of ethyl acetate and centrifuged; supernatants were combined and then evaporated to dryness on a vacuum concentrator (Eppendorf). The residues were resuspended in 0.5 ml of 70% methanol (v/v) and centrifuged to clarify phases. Following centrifugation, the supernatants were pipetted to glass vials and then analysed with HPLC-MS/MS (LCMS-8040 system, Shimadzu).

(e) Benzoxazinoid extraction and quantification

Approximately 100 mg of frozen leaf powder was suspended with 1 ml of the extraction solution (50% methanol containing 0.5% formic acid; all in volume) in 1.5 ml Eppendorf tubes and vortexed vigorously for 10 min. The samples were centrifuged at 13 000 *g* for 15 min, and 450 µl of the supernatants were transferred to glass vials for analysis on a HPLC-MS/MS system (LCMS-8040, Shimadzu) according to a previously published method [36].

(f) RNA extraction and data analysis

RNA extraction and gene expression analyses were performed as described previously [57,58]. Total RNA was extracted from ground leaf samples using the TRIzol reagent (Invitrogen) and RNA quality, purity and concentration were assessed using a spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific).

RNA-seq was performed at 5 G depth on a HiSeq2500-PE125 platform (Illumina). The resulting sequences were trimmed based on quality scores and mapped to the maize B73 reference genome sequence V3 and maize working gene set V5a using the following modifications from default parameters: maximum intron size, 100 000; minimum intron size, 20; up to two

mismatches allowed [59]. We used Tophat and Cufflinks to assemble the transcripts and to identify DEGs. Transcripts with a false discovery rate (FDR) less than 0.05 and an absolute value of log2 (FPKM of induced/FPKM of control) greater than or equal to 1 were selected as DEGs for further analysis [60]. The expression levels of genes were normalized using the number of reads per kb of exon sequence in a gene per million mapped reads.

(g) Transcription factor and promoter analysis

Gene IDs of TFs in maize were retrieved from Plant Transcription Factor Database [49], and were then searched against the DEGs in systemic leaves to obtain the systemically regulated TFs. To identify the TFs in silico that may bind to promoters of Bx biosynthetic genes, 2 kb sequences upstream of the start codons of Bx biosynthesis genes were retrieved from the Phytozome database, and the TF binding sites within these sequences were predicted using the TFBS prediction tool [49].

(h) Data analysis

Venn diagrams were generated using the Venny 2.1.0 drawing software (http://bioinfogp.cnb.csic.es/tools/venny/index.html) Student's *t*-test was used to determine the differences between different treatment groups.

Data accessibility. The raw sequence data are deposited in NCBI's Short Read Archive under the BioProject ID (PRJNA485551).

Authors' contributions. J.W., C.H. and J.Q designed research; S.U.M., J.Q., C.Z., L.W. and J.Z. performed research; S.U.M. C.L., J.L. and Y.X. analysed data; S.U.M., C.H. and J.W. wrote the paper and S.U.M made the illustrations.

Competing interests. We declare we have no competing interests.

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References

1. Agrawal AA. 1998 Induced responses to herbivory and increased plant performance. *Science* **279**, 1201–1202. (doi:10.1126/science.279.5354.1201)
2. Karban R. 2011 The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* **25**, 339–347. (doi:10.1111/j.1365-2435.2010.01789.x)
3. Zavala JA, Patankar AG, Gase K, Baldwin IT. 2004 Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. *Proc. Natl Acad. Sci. USA* **101**, 1607–1612. (doi:10.1073/pnas.0305096101)
4. Baldwin IT. 1998 Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl Acad. Sci. USA* **95**, 8113–8118. (doi:10.1073/pnas.95.14.8113)
5. Tor M, Lotze MT, Holton N. 2009 Receptor-mediated signalling in plants: molecular patterns and programmes. *J. Exp. Bot.* **60**, 3645–3654. (doi:10.1093/jxb/erp233)
6. Shan XY, Wang ZL, Xie D. 2007 Jasmonate signal pathway in *Arabidopsis*. *J. Integr. Plant Biol.* **49**, 81–86. (doi:10.1111/j.1672-9072.2007.00416.x)
7. Erb M, Meldau S, Howe GA. 2012 Role of phytohormones in insect-specific plant reactions. *Trends Plant Sci.* **17**, 250–259. (doi:10.1016/j.tplants.2012.01.003)
8. Staswick PE. 2002 Jasmonate response locus *JAR1* and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. *Plant Cell* **14**, 1405–1415. (doi:10.1105/tpc.000885)
9. Urano K, Maruyama K, Jikumaru Y, Kamiya Y, Yamaguchi-Shinozaki K, Shinozaki K. 2017 Analysis of plant hormone profiles in response to moderate dehydration stress. *Plant J.* **90**, 17–36. (doi:10.1111/tpj.13460)
10. Erb M, Robert CAM, Turlings TCJ. 2011 Induction of root-resistance by leaf-herbivory follows a vertical gradient. *J. Plant Interact* **6**, 133–136. (doi:10.1080/17429145.2010.545958)
11. Wu JQ, Baldwin IT. 2010 New insights into plant responses to the attack from insect herbivores. *Annu. Rev. Genet.* **44**, 1–24. (doi:10.1146/annurev-genet-102209-163500)
12. Christensen SA *et al.* 2013 The maize lipoxygenase, *ZmLOX10*, mediates green leaf volatile, jasmonate and herbivore-induced plant volatile production for defense against insect attack. *Plant J.* **74**, 59–73. (doi:10.1111/tpj.12101)
13. Heil M, Ton J. 2008 Long-distance signalling in plant defence. *Trends Plant Sci.* **13**, 264–272. (doi:10.1016/j.tplants.2008.03.005)
14. Bozorov TA, Dinh ST, Baldwin IT. 2017 JA but not JA–Ile is the cell-nonautonomous signal activating JA mediated systemic defenses to herbivory in *Nicotiana attenuata*. *J. Integr. Plant Biol.* **59**, 552–571. (doi:10.1111/jipb.12545)
15. Howe GA, Lightner J, Browse J, Ryan CA. 1996 An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* **8**, 2067–2077. (doi:10.1105/tpc.8.11.2067)
16. Stintzi A, Weber H, Reymond P, Browse J, Farmer EE. 2001 Plant defense in the absence of jasmonic acid: the role of cyclopentenones. *Proc. Natl Acad. Sci. USA* **98**, 12 837–12 842. (doi:10.1073/pnas.211311098)
17. Halitschke R, Baldwin IT. 2003 Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. *Plant J.* **36**, 794–807. (doi:10.1046/j.1365-313X.2003.01921.x)
18. Kang JH, Wang L, Giri A, Baldwin IT. 2006 Silencing threonine deaminase and *JAR4* in *Nicotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta*. *Plant Cell* **18**, 3303–3320. (doi:10.1105/tpc.106.041103)
19. Paschold A, Halitschke R, Baldwin IT. 2007 Co(i)-ordinating defenses: NaCOI1 mediates herbivore-induced resistance in *Nicotiana attenuata* and reveals the role of herbivore movement in avoiding defenses. *Plant J.* **51**, 79–91. (doi:10.1111/j.1365-313X.2007.03119.x)
20. Schweizer F *et al.* 2013 *Arabidopsis* basic helix-loop-helix transcription factors MYC2, MYC3, and MYC4 regulate glucosinolate biosynthesis, insect performance, and feeding behavior. *Plant Cell* **25**, 3117–3132. (doi:10.1105/tpc.113.115139)
21. Erb M, Flors V, Karlen D, de Lange E, Planchamp C, D'Alessandro M, Turlings TCJ, Ton J. 2009 Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *Plant J.* **59**, 292–302. (doi:10.1111/j.1365-313X.2009.03868.x)
22. Perkins LE, Cribb BW, Brewer PB, Hanan J, Grant M, de Torres M, Zalucki MP. 2013 Generalist insects behave in a jasmonate-dependent manner on their host plants, leaving induced areas quickly and staying longer on distant parts. *Proc. R. Soc. B* **280**, 20122646. (doi:10.1098/rspb.2012.2646)
23. Stork W, Diezel C, Halitschke R, Galis I, Baldwin IT. 2009 An ecological analysis of the herbivory-elicited JA burst and its metabolism: plant memory processes and predictions of the moving target model. *PLoS ONE* **4**, e4697. (doi:10.1371/journal.pone.0004697)
24. Green TR, Ryan CA. 1972 Wound-induced proteinase inhibitor in plant leaves—possible defense mechanism against insects. *Science* **175**, 776–777. (doi:10.1126/science.175.4023.776)
25. Mousavi SAR, Chauvin A, Pascaud F, Kellenberger S, Farmer EE. 2013 GLUTAMATE RECEPTOR-LIKE genes mediate leaf-to-leaf wound signalling. *Nature* **500**, 422–426. (doi:10.1038/nature12478)
26. Glauser G, Grata E, Dubugnon L, Rudaz S, Farmer EE, Wolfender JL. 2008 Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *J. Biol.*

Chem. **283**, 16 400–16 407. (doi:10.1074/jbc.M801760200)

27. Schittko U, Preston CA, Baldwin IT. 2000 Eating the evidence? *Manduca sexta* larvae can not disrupt specific jasmonate induction in *Nicotiana attenuata* by rapid consumption. *Planta* **210**, 343–346. (doi:10.1007/PI00008143)
28. Niemeyer HM. 2009 Hydroxamic acids derived from 2-hydroxy-2 h-1,4-benzoxazin-3(4 h)-one: key defense chemicals of cereals. *J. Agric. Food Chem.* **57**, 1677–1696. (doi:10.1021/jf8034034)
29. Morant AV, Jorgensen K, Jorgensen C, Paquette SM, Sanchez-Perez R, Moller BL, Bak S. 2008 β-glucosidases as detonators of plant chemical defense. *Phytochemistry* **69**, 1795–1813. (doi:10.1016/j.phytochem.2008.03.006)
30. Meihls LN *et al.* 2013 Natural variation in maize aphid resistance is associated with 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one glucoside methyltransferase activity. *Plant Cell* **25**, 2341–2355. (doi:10.1105/tpc.113.112409)
31. Handrick V *et al.* 2016 Biosynthesis of 8-*O*-methylated benzoxazinoid defense compounds in maize. *Plant Cell* **28**, 1682–1700. (doi:10.1105/tpc.16.00065)
32. Oikawa A, Ishihara A, Hasegawa M, Kodama O, Iwamura H. 2001 Induced accumulation of 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one glucoside (HDMBOA-Glc) in maize leaves. *Phytochemistry* **56**, 669–675. (doi:10.1016/S0031-9422(00)00494-5)
33. Oikawa A, Ishihara A, Iwamura H. 2002 Induction of HDMBOA-Glc accumulation and DIMBOA-Glc 4-*O*-methyltransferase by jasmonic acid in poaceous plants. *Phytochemistry* **61**, 331–337. (doi:10.1016/S0031-9422(02)00225-X)
34. Oikawa A, Ishihara A, Tanaka C, Mori N, Tsuda M, Iwamura H. 2004 Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves. *Phytochemistry* **65**, 2995–3001. (doi:10.1016/j.phytochem.2004.09.006)
35. Qi J, Malook SU, Shen G, Gao L, Zhang C, Li J, Zhang J, Wang L, Wu J. 2018 Current understanding of maize and rice defense against insect herbivores. *Plant Divers.* **40**, 189–195. (doi:10.1016/j.pld.2018.06.006)
36. Qi J *et al.* 2016 Oral secretions from *Mythimna separata* insects specifically induce defence responses in maize as revealed by high-dimensional biological data. *Plant Cell Environ.* **39**, 1749–1766. (doi:10.1111/pce.12735)
37. Engelberth J, Seidl-Adams I, Schultz JC, Tumlinson JH. 2007 Insect elicitors and exposure to green leafy volatiles differentially upregulate major octadecanoids and transcripts of 12-oxo phytodienoic acid reductases in *Zea mays*. *Mol. Plant Microbe Interact.* **20**, 707–716. (doi:10.1094/Mpmi-20-6-0707)
38. Engelberth J, Contreras CF, Viswanathan S. 2012 Transcriptional analysis of distant signaling induced by insect elicitors and mechanical wounding in *Zea mays*. *PLoS ONE* **7**, e34855. (doi:10.1371/journal.pone.0034855)
39. Erb M, Robert CAM, Hibbard BE, Turlings TCJ. 2011 Sequence of arrival determines plant-mediated interactions between herbivores. *J. Ecol.* **99**, 7–15. (doi:10.1111/j.1365-2745.2010.01757.x)
40. Paschold A, Bonaventure G, Kant MR, Baldwin IT. 2008 Jasmonate perception regulates jasmonate biosynthesis and JA–Ile metabolism: the case of COI1 in *Nicotiana attenuata*. *Plant Cell Physiol.* **49**, 1165–1175. (doi:10.1093/pcp/pcn091)
41. Howe GA, Jander G. 2008 Plant immunity to insect herbivores. *Annu. Rev. Plant Biol.* **59**, 41–66. (doi:10.1146/annurev.arplant.59.032607.092825)
42. Glauser G, Marti G, Villard N, Doyen GA, Wolfender JL, Turlings TCJ, Erb M. 2011 Induction and detoxification of maize 1,4-benzoxazin-3-ones by insect herbivores. *Plant J.* **68**, 901–911. (doi:10.1111/j.1365-313X.2011.04740.x)
43. Frey M *et al.* 1997 Analysis of a chemical plant defense mechanism in grasses. *Science* **277**, 696–699. (doi:10.1126/science.277.5326.696)
44. Frey M, Schullehner K, Dick R, Fiesselmann A, Gierl A. 2009 Benzoxazinoid biosynthesis, a model for evolution of secondary metabolic pathways in plants. *Phytochemistry* **70**, 1645–1651. (doi:10.1016/j.phytochem.2009.05.012)
45. Pechan T, Ye LJ, Chang YM, Mitra A, Lin L, Davis FM, Williams WP, Luthe DS. 2000 A unique 33-kD cysteine proteinase accumulates in response to larval feeding in maize genotypes resistant to fall armyworm and other lepidoptera. *Plant Cell* **12**, 1031–1040. (doi:10.1105/tpc.12.7.1031)
46. Ankala A, Luthe DS, Williams WP, Wilkinson JR. 2009 Integration of ethylene and jasmonic acid signaling pathways in the expression of maize defense protein Mir1-CP. *Mol. Plant Microbe Interact.* **22**, 1555–1564. (doi:10.1094/MPMI-22-12-1555)
47. Wouters FC, Blanchette B, Gershenzon J, Vassao DG. 2016 Plant defense and herbivore counter-defense: benzoxazinoids and insect herbivores. *Phytochem. Rev.* **15**, 1127–1151. (doi:10.1007/s11101-016-9481-1)
48. Wouters FC, Gershenzon J, Vassão DG. 2016 Benzoxazinoids: reactivity and modes of action of a versatile class of plant chemical defenses. *J. Braz. Chem. Soc.* **27**, 1379–1397. (doi:10.5935/0103-5053.20160177)
49. Jin J, Tian F, Yang DC, Meng YQ, Kong L, Luo J, Gao G. 2017 PlantTFDB 4.0: toward a central hub for transcription factors and regulatory interactions in plants. *Nucleic Acids Res.* **45**, D1040–D1045. (doi:10.1093/nar/gkw982)
50. Maag D, Kohler A, Robert CA, Frey M, Wolfender JL, Turlings TC, Glauser G, Erb M. 2016 Highly localized and persistent induction of Bx1-dependent herbivore resistance factors in maize. *Plant J.* **88**, 976–991. (doi:10.1111/tpj.13308)
51. Tzin V, Susan YH, Strickler R, Bartsch LJ, Archer CM. 2017 Rapid defense responses in maize leaves induced by *Spodoptera exigua* caterpillar feeding. *J. Exp. Bot.* **28**, 4709–4723. (doi:10.1093/jxb/erx274)
52. Wu J, Hettenhausen C, Meldau S, Baldwin IT. 2007 Herbivory rapidly activates MAPK signaling in attacked and unattacked leaf regions but not between leaves of *Nicotiana attenuata*. *Plant Cell* **19**, 1096–1122. (doi:10.1105/tpc.106.049353)
53. Hettenhausen C, Heinrich M, Baldwin IT, Wu JQ. 2014 Fatty acid-amino acid conjugates are essential for systemic activation of salicylic acid-induced protein kinase and accumulation of jasmonic acid in *Nicotiana attenuata*. *BMC Plant Biol.* **14**, 326. (doi:10.1186/S12870-014-0326-Z)
54. Hettenhausen C *et al.* 2017 Stem parasitic plant *Cuscuta australis* (dodder) transfers herbivory-induced signals among plants. *Proc. Natl Acad. Sci. USA* **114**, E6703–E6709. (doi:10.1073/pnas.1704536114)
55. Koo AJK, Gao XL, Jones AD, Howe GA. 2009 A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. *Plant J.* **59**, 974–986. (doi:10.1111/j.1365-313X.2009.03924.x)
56. Glauser G, Dubugnon L, Mousavi SAR, Rudaz S, Wolfender JL, Farmer EE. 2009 Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded *Arabidopsis*. *J. Biol. Chem.* **284**, 34 506–34 513. (doi:10.1074/jbc.M109.061432)
57. Balmer D, de Papajewski DV, Planchamp C, Glauser G, Mauch-Mani B. 2013 Induced resistance in maize is based on organ-specific defence responses. *Plant J.* **74**, 213–225. (doi:10.1111/tpj.12114)
58. Song J *et al.* 2017 Transcriptomics and alternative splicing analyses reveal large differences between maize lines b73 and mo17 in response to aphid *Rhopalosiphum padi* infestation. *Front. Plant Sci.* **8**, 1738. (doi:10.3389/fpls.2017.01738)
59. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL. 2013 TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* **14**, R36. (doi:10.1186/gb-2013-14-4-r36)
60. Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, Salzberg SL, Wold BJ, Pachter L. 2010 Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* **28**, 511–515. (doi:10.1038/nbt.1621)