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

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Maize (*Zea mays*) is a staple cereal crop cultivated all over the world, which is threatened by various insects. Feeding of the lepidopteran insect *Mythimna separata* triggers defense signaling and increases anti-herbivore Benzoxazinoids (Bxs) in the insect-damaged maize leaves. However, the herbivory-elicited within-leaf and leaf-to-leaf systemic signaling in maize remains largely unexplored. Here we show that simulated *M. separata* herbivory and mechanical wounding elicited increased levels of Jasmonic Acid (JA) and 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 systemic leaf, and consistently, this leaf exhibited increased

defense 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 coexpression 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 existed the local leaves within 30 min after elicitation. This study provides new insight into the temporal and spatial regulation of defense responses of maize against lepidopteran insects.

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