Figures and figure supplements

Sequestration and activation of plant toxins protect the western corn rootworm from enemies at multiple trophic levels

Christelle AM Robert et al
Figure 1. *Diabrotica virgifera* specifically and actively sequesters maize benzenoxazinoids (BXs) (Figure 1—figure supplements 1–3). (A) BX concentration in larvae of the specialist *D. virgifera*, and the generalists *D. undecimpunctata* (*D. undecim*.) and *D. balteata*. Numbers denote the six most abundant BXs. Stars indicate significant differences between species (one-way ANOVA on transformed data (rank and square root transformations), *p*<0.05). (B) BX concentrations in the haemolymph, gut, muscles, exudates (surface), and frass of *D. virgifera* larvae fed on wild-type B73 plants. (C) Correlation between BX concentrations in maize B73 plants and in third instar *D. virgifera* larvae that fed on those plants since hatching. Unlabeled blue dots correspond to other types of BXs. A linear regression between plant and larval concentrations is shown (R² = 0.8141, p=0.004, excl. MBOA-Glc and HDMBOA-Glc). Means ± SE are shown. Raw data are available in Figure 1—source data 1.

DOI: https://doi.org/10.7554/eLife.29307.003

Robert et al. eLife 2017;6:e29307. DOI: https://doi.org/10.7554/eLife.29307
Figure 1—figure supplement 1. Benzoxazinoid levels in Diabrotica virgifera larvae fed on different maize lines. H88 corresponds to the wild type, 428G to the original bx1 mutant, 24R to an igl mutant and 32R to a 100% BX-deficient bx1.igl double mutant (see [29]). Means ± SE are shown. Letters indicate significant differences between genotypes (one-way ANOVAs, p<0.05). No BXs were detected in larvae fed on 32R.

DOI: https://doi.org/10.7554/eLife.29307.004
**Figure 1**

Larvae were exposed to different plant tissues (B73 and bx1) and the concentration of DIMBOA, HDMBOA-Glc, DIM2BOA-Glc, and MBOA-Glc was measured. Significant differences are indicated by letters (a, b, c). The concentration is expressed as µg/g FW. Differences were analyzed using ANOVA with Tukey’s multiple comparison test.

- **DIMBOA**: Differences were observed between plant tissues and larvae, with B73 and bx1 showing different concentrations.
- **HDMBOA-Glc**: Significant differences were found for the interaction between plant tissue and larvae, with B73 showing higher concentrations than bx1.
- **DIM2BOA-Glc**: Differences were observed between plant tissues and larvae, with B73 showing higher concentrations than bx1.
- **MBOA-Glc**: No significant differences were observed between plant tissues and larvae.

**Organic Carbon (Org)** and **Genomic DNA (Gen)** levels were also measured, with no significant differences observed.

*P-values:***

- DIMBOA: Gen: ***
- HDMBOA-Glc: Org: ***
- DIM2BOA-Glc: Org: ***
- MBOA-Glc: Gen:**
Figure 1—figure supplement 2. Benzoxazinoid levels in Diabrotica virgifera larvae fed wild-type (B73) and bx1 (bx1:B73) mutant plants. Means ± SE are shown. Results of two-way ANOVAs are shown (*p<0.05, **p<0.01, ***p<0.001). Letters indicate significant differences between genotypes (post-hoc test, p<0.05).

DOI: https://doi.org/10.7554/eLife.29307.005
**Figure 1—figure supplement 3.** Benzoxazinoid levels in aqueous surface extracts of *Diabrotica virgifera* larvae fed on wild-type (B73) and bx1 (bx1: B73) mutant plants. Means ± SE are shown. Stars indicate significant differences between genotypes (Student t-test, p<0.001).

DOI: https://doi.org/10.7554/eLife.29307.006
Figure 2. Stabilization and reactivation of stored benzoazinoids (BXs) by Diabrotica virgifera and its natural enemies (Figure 2—figure supplement 1). (A) Stabilization of MBOA by conversion to MBOA-Glc in D. virgifera gut extracts. (B) HDMBOA-Glc deglucosylation in D. virgifera gut extracts. (C) BX reactivation in D. virgifera larvae upon mechanical tissue disruption. (D) BX reactivation in D. virgifera larvae upon exposure to the entomopathogenic nematode (EPN) Heterorhabditis bacteriophora. (E) BX reactivation by H. bacteriophora 24 hr after addition of purified metabolites. (F) BX reactivation by the EPN endosymbiotic bacterium Photorhabdus luminescens 24 hr after addition of purified metabolites. Means ± SE are shown. Stars indicate significant differences between time points (repeated measures ANOVAs, A–C) or between treatments (Student’s t-tests, D–F; *p<0.05, **p<0.01, ***p<0.001). Raw data are available in Figure 2—source data 1.

DOI: https://doi.org/10.7554/eLife.29307.008
Figure 2—figure supplement 1. Degradation of MBOA-Glc by plant-derived hydrolases. MBOA-Glc concentrations upon incubation with a broad-spectrum almond glucosidase (CAS Nr. 9001-22-3) and with root extracts of a benzoxazinoid-free maize seedling (32R). Means ± SE are shown. No significant change in MBOA-Glc was observed (one-way ANOVA).

DOI: https://doi.org/10.7554/eLife.29307.009
Figure 3. Benzoxazinoids (BXs) protect Diabrotica virgifera from its natural enemies (Figure 3—figure supplement 1). (A) Infection success by the entomopathogenic nematode (EPN) Heterorhabditis bacteriophora on D. virgifera larvae fed on WT (B73 and W22) or BX-deficient (bx1:B73, bx1:W22, bx2:W22) plants. (B) Effect of 7 days exposure to BXs on H. bacteriophora infectivity. (C) Effect of 7 days exposure to BXs on H. bacteriophora mortality. (D) Effect of BXs on the growth of the symbiotic entomopathogenic bacterium Photorhabdus luminescens. Different letters indicate significant differences between plant genotypes. Means ± SE are shown. Stars indicate significant differences between concentrations (A-C: one-way ANOVA, D: repeated measures ANOVA, *p<0.05, **p<0.01, ***p<0.001). Raw data are available in Figure 3—source data 1.

DOI: https://doi.org/10.7554/eLife.29307.011
Robert et al. eLife 2017;6:e29307. DOI: https://doi.org/10.7554/eLife.29307

10 of 13
Figure 3—figure supplement 1. Growth curves and growth characteristics of Photorhabdus luminescens EN01 upon exposure to MBOA-Glc, HDMBOA-Glc and MBOA at different concentrations. Means ± SE are shown. Stars indicate significant differences between genotypes (repeated measures ANOVAs, p<0.05).

DOI: https://doi.org/10.7554/eLife.29307.012
Figure 4. MBOA-Glc decreases the attractiveness of Diabrotica virgifera larvae. (A) Attraction of the entomopathogenic nematode (EPN) Heterorhabditis bacteriophora to D. virgifera larvae fed on wild-type (B73) and bx1-mutant (bx1:B73) (top) and aqueous surface extracts of larvae fed on wild type and bx1-mutant (bottom). (B) H. bacteriophora attraction to pure MBOA-Glc and HDMBOA-Glc at physiological concentrations. Means ± SE are shown. Letters indicate significant differences between treatments (one sample t-tests, *p<0.05, **p<0.01, ***p<0.001). Raw data are available in Figure 4—source data 1.

DOI: https://doi.org/10.7554/eLife.29307.014
Figure 5. A model illustrating how BX sequestration and activation of plant toxins protects Diabrotica virgifera larvae from their enemies at multiple levels. MBOA-Glc, released in the frass and on the exoskeleton, repels infective juvenile entomopathogenic nematodes. Upon infection by nematodes and their symbiotic entomopathogenic bacteria, HDMBOA-Glc is activated to produce MBOA. Both HDMBOA-Glc and the activated MBOA reduce the growth of the symbiotic bacteria and kill EPNs.

DOI: https://doi.org/10.7554/eLife.29307.016