Figures and figure supplements

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

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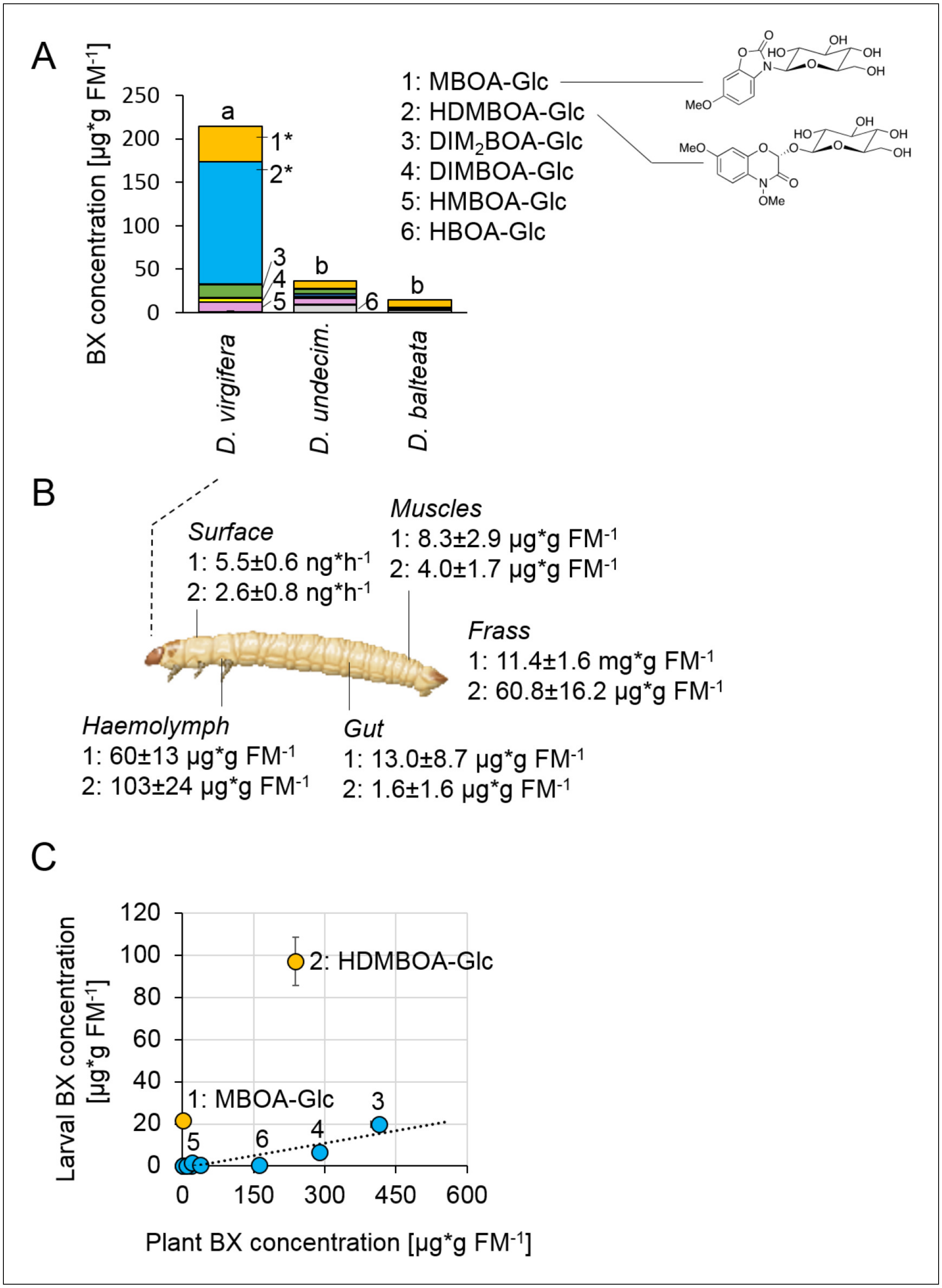

Figure 1. *Diabrotica virgifera* specifically and actively sequesters maize benzoxazinoids (BXs) (***Figure 1—figure supplements 1–3***). (**A**) BX concentration in larvae of the specialist *D. virgifera*, and the generalists *D. undecimpunctata* (*D.undecim.*) and *D. balteata*. Numbers denote the 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 (R^2 = 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***.

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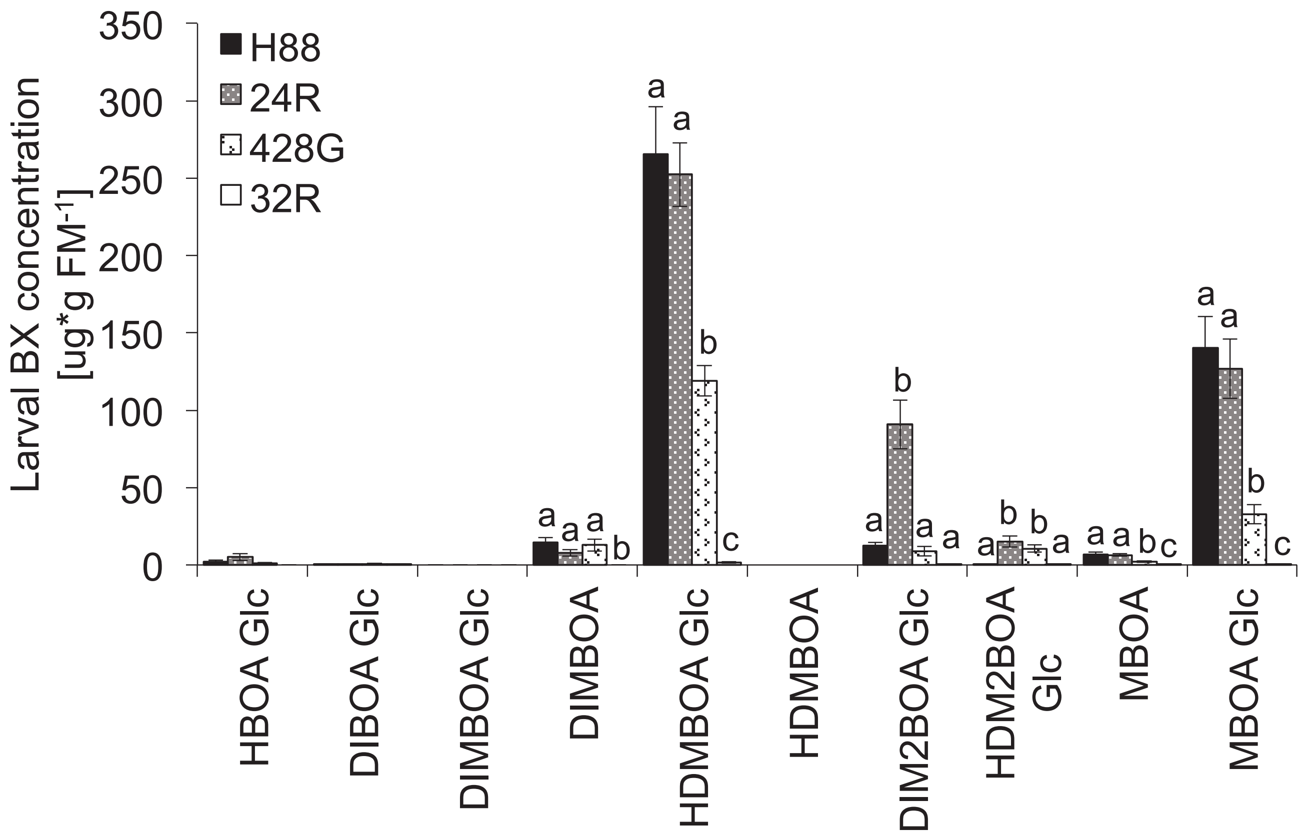

Figure 1—figure supplement 1. Benzoxazinoid levels in *Diabrotica virgifera* larvae fed on different maize lines. H88 corresponds to the wild type, 428G to the original *bx1* mutant, 24R to an *igl* mutant and 32R to a 100% BX-deficient *bx1.igl* double mutant (see [29]). Means ± SE are shown. Letters indicate significant differences between genotypes (one-way ANOVAs, p<0.05). No BXs were detected in larvae fed on 32R.
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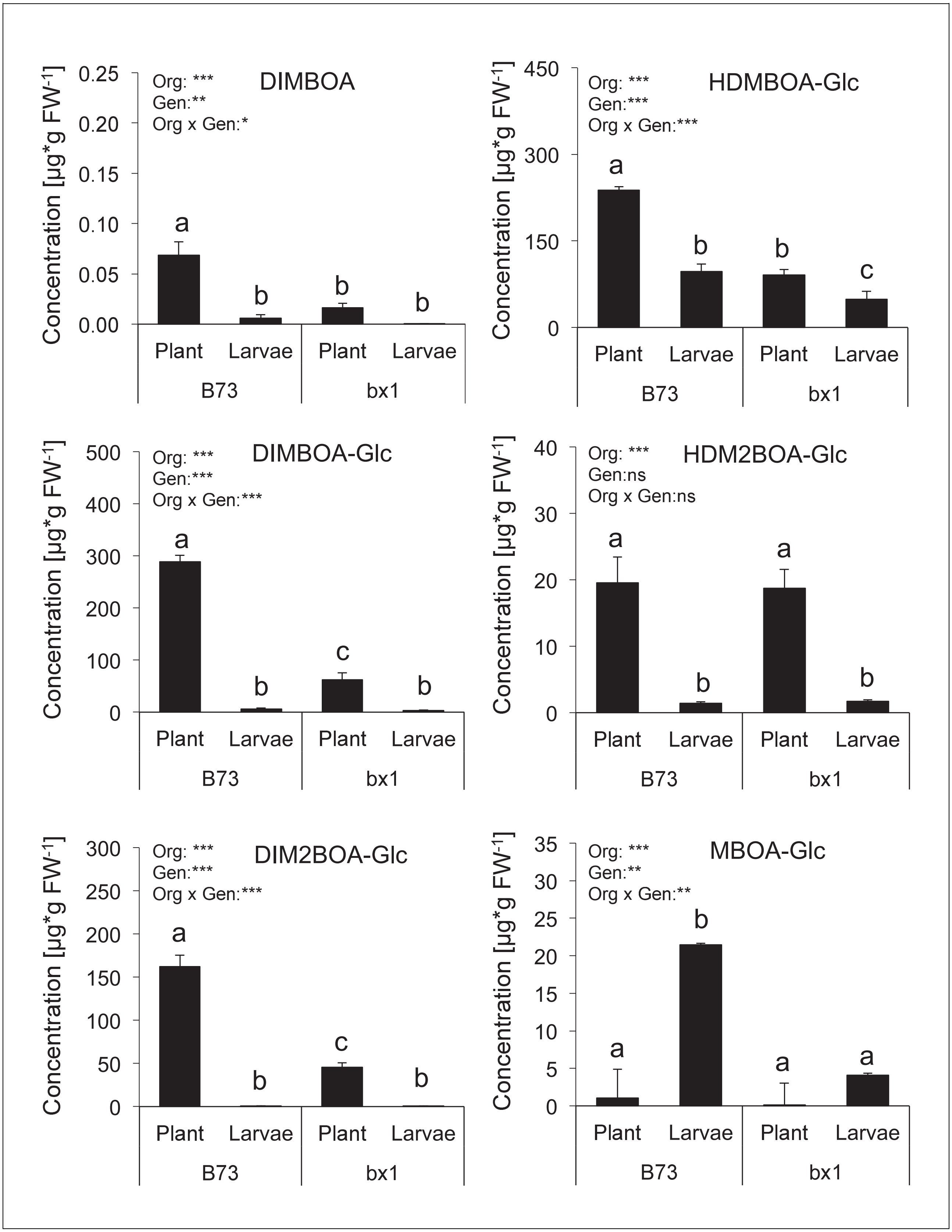
DIMBOA
Org: ***
Gen:**
Org x Gen:*
Concentration [µg*g FW-1]
0.25
0.20
0.15
0.10
0.05
0.00
a
b
b
b
Plant
Larvae
Plant
Larvae
B73
bx1
HDMBOA-Glc
Org: ***
Gen:***
Org x Gen:***
450
300
150
0
a
b
b
c
DIMBOA-Glc
Org: ***
Gen:***
Org x Gen:***
500
400
300
200
100
0
a
b
c
b
HDM2BOA-Glc
Org: ***
Gen:ns
Org x Gen:ns
40
30
20
10
0
a
b
a
b
DIM2BOA-Glc
Org: ***
Gen:***
Org x Gen:***
300
250
200
150
100
50
0
a
b
c
b
MBOA-Glc
Org: ***
Gen:**
Org x Gen:**
35
30
25
20
15
10
5
0
a
b
a
a

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).

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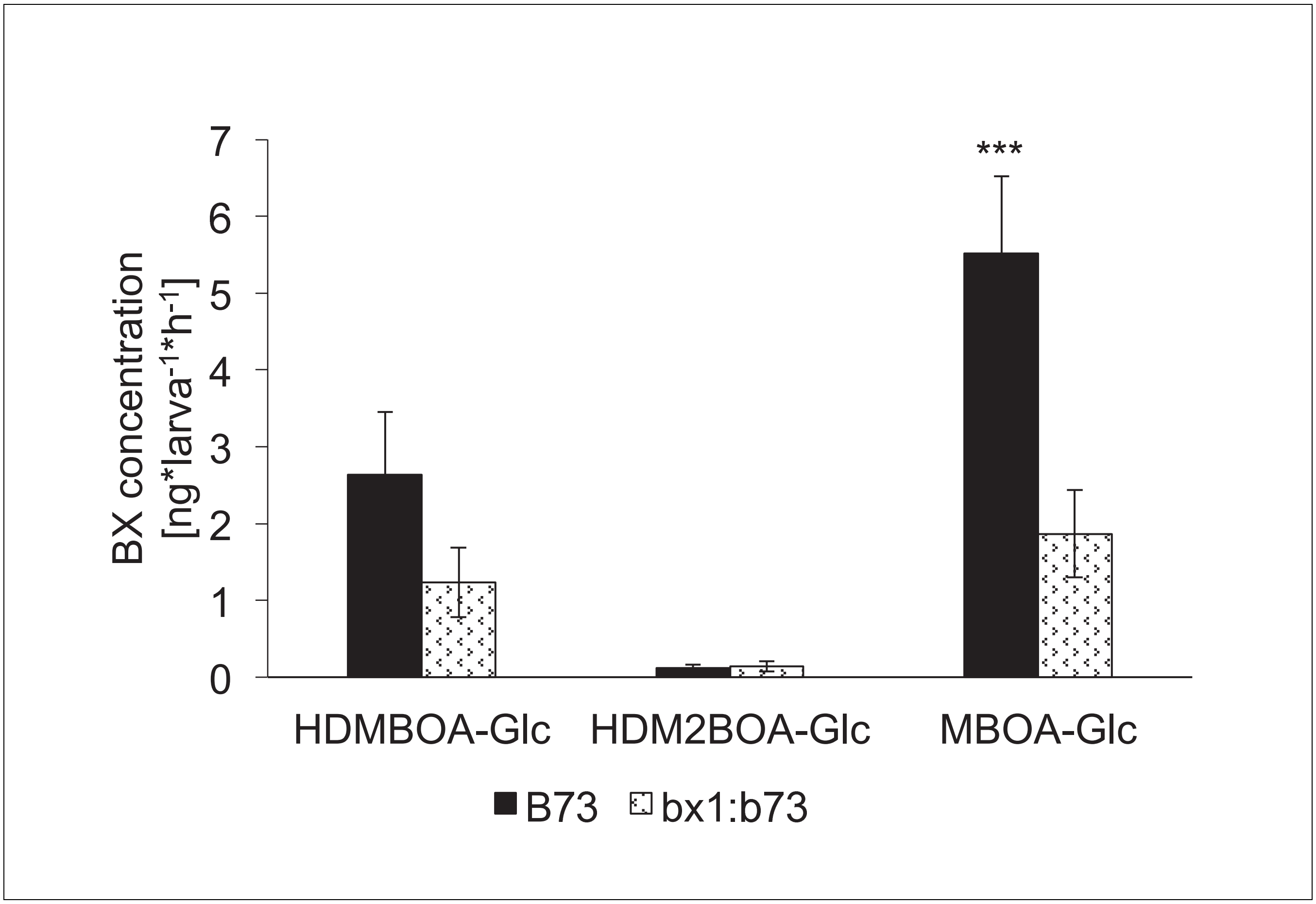

Figure 1—figure supplement 3. Benzoxazinoid levels in aqueous surface extracts of *Diabrotica virgifera* larvae fed on wild-type (B73) and *bx1* (bx1: B73) mutant plants. Means ± SE are shown. Stars indicate significant differences between genotypes (Student t-test, $p<0.001$).

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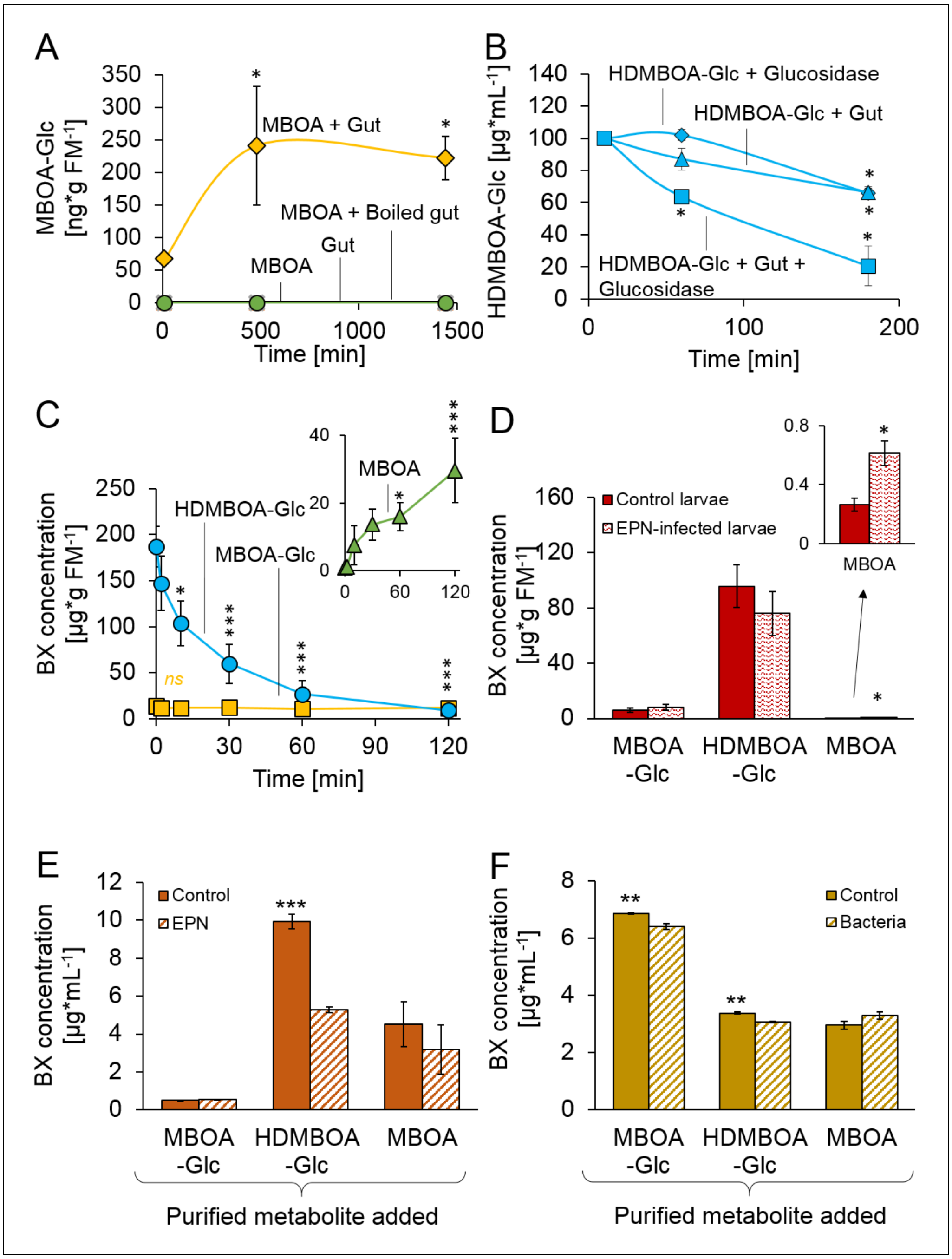

Figure 2. Stabilization and reactivation of stored benzoxazinoids (BXs) by *Diabrotica virgifera* and its natural enemies (***Figure 2—figure supplement 1***). (**A**) Stabilization of MBOA by conversion to MBOA-Glc in *D. virgifera* gut extracts. (**B**) HDMBOA-Glc deglucosylation in *D. virgifera* 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***.

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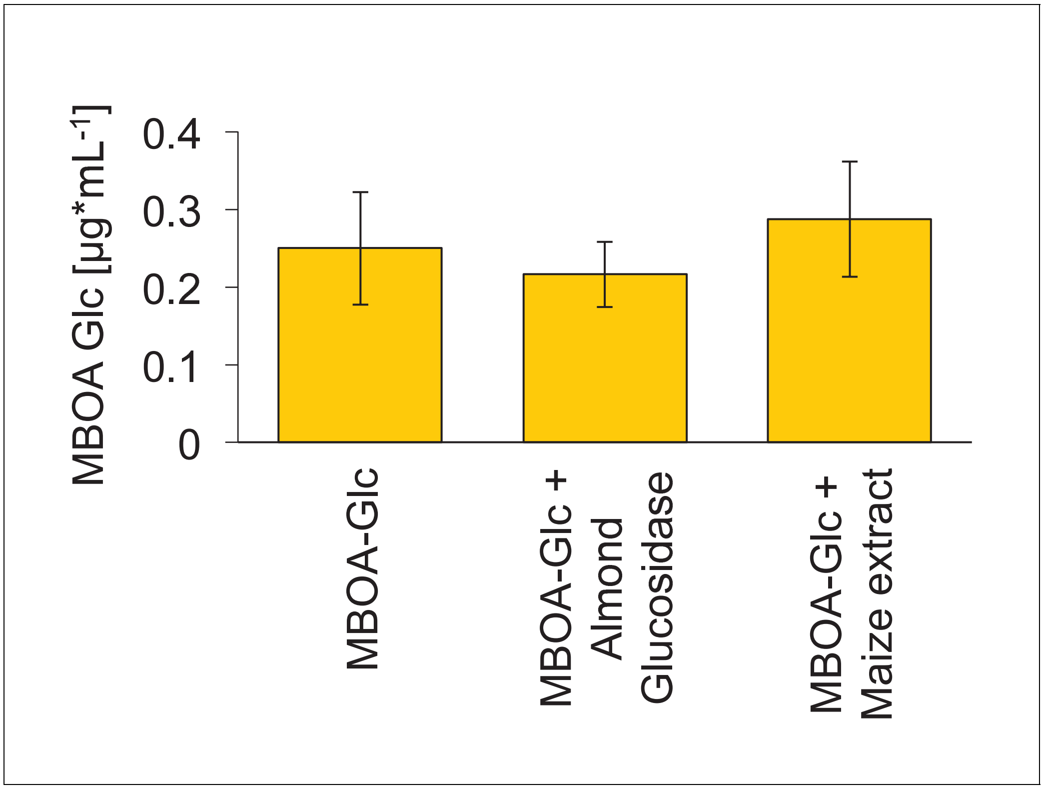

Figure 2—figure supplement 1. Degradation of MBOA-Glc by plant-derived hydrolases. MBOA-Glc concentrations upon incubation with a broad-spectrum almond glucosidase (CAS Nr. 9001-22-3) and with root extracts of a benzoxazinoid-free maize seedling (32R). Means ± SE are shown. No significant change in MBOA-Glc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

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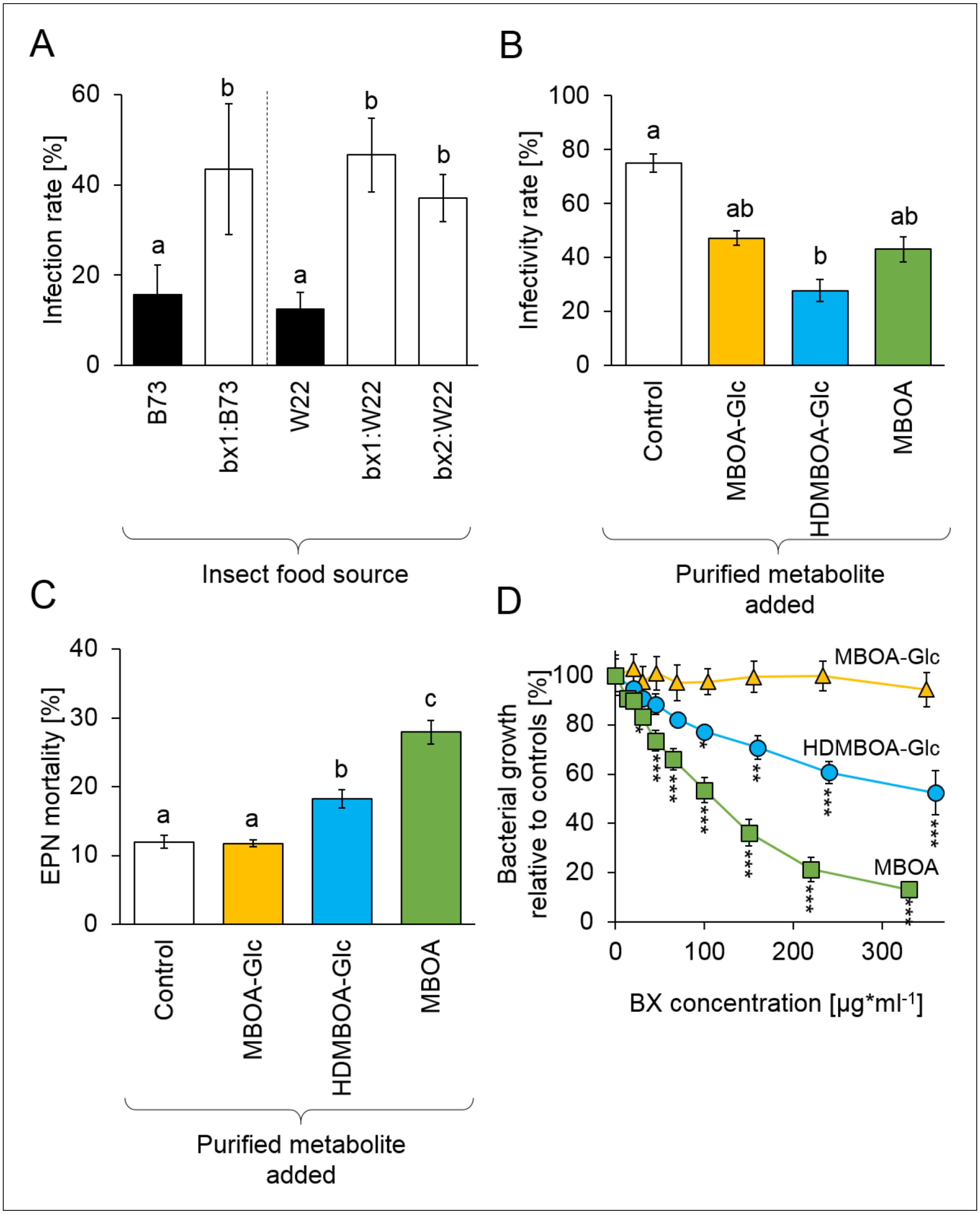

Figure 3. Benzoxazinoids (BXs) protect *Diabrotica virgifera* from its natural enemies (***Figure 3—figure supplement 1***). (**A**) Infection success by the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora* on *D. virgifera* larvae fed on WT (B73 and W22) or BX-deficient (bx1:B73, bx1:W22, 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***.
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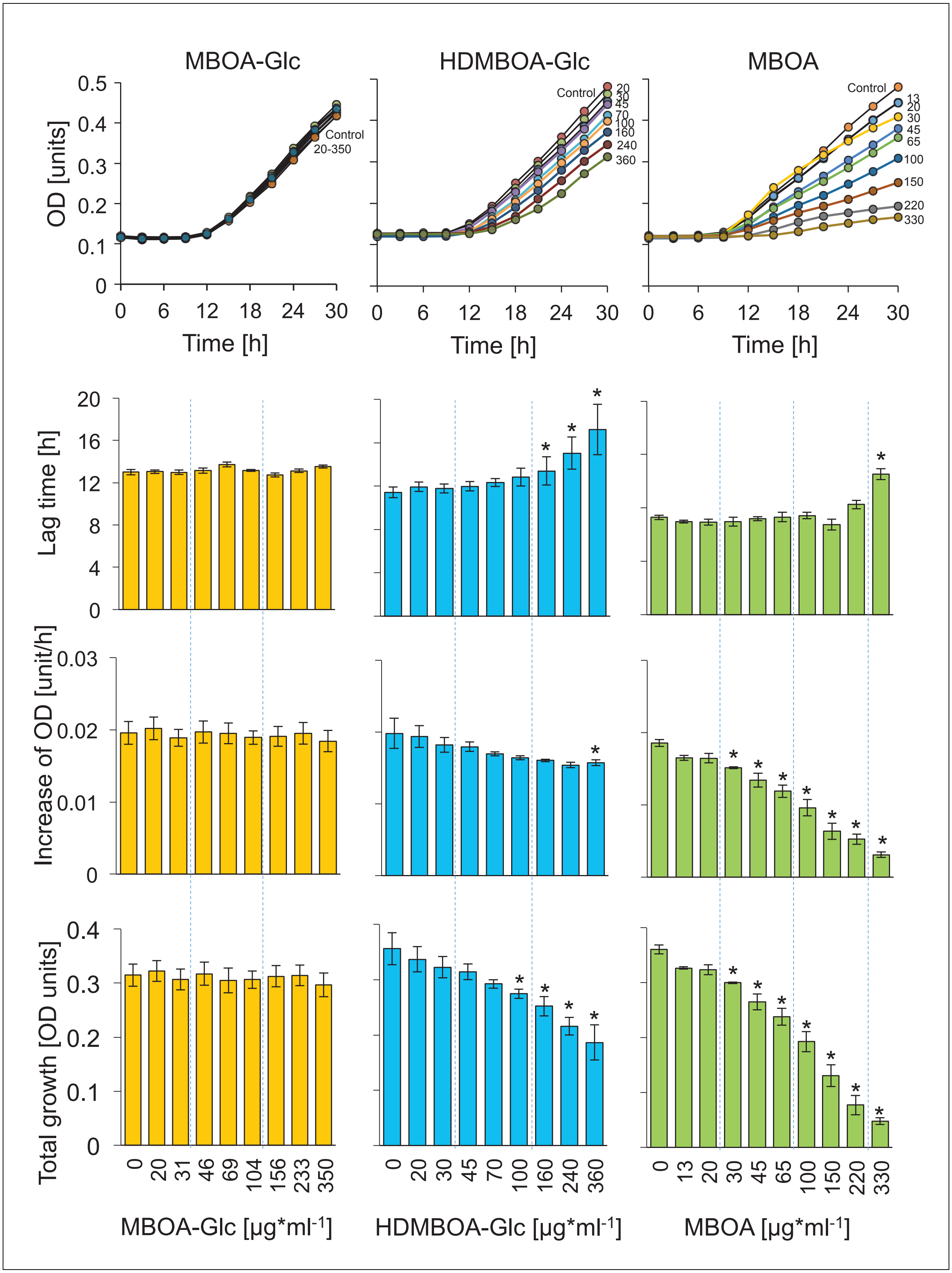
MBOA-Glc
HDMBOA-Glc
MBOA
OD [units]
Time [h]
Control
20-350
Control
20
30
45
70
100
160
240
360
Control
13
20
30
45
65
100
150
220
330
Lag time [h]
Increase of OD [unit/h]
Total growth [OD units]
0 20 31 46 69 104 156 233 350
0 20 30 45 70 100 160 240 360
0 13 20 30 45 65 100 150 220 330
MBOA-Glc [µg*ml-1]
HDMBOA-Glc [µg*ml-1]
MBOA [µg*ml-1]

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, $p<0.05$).
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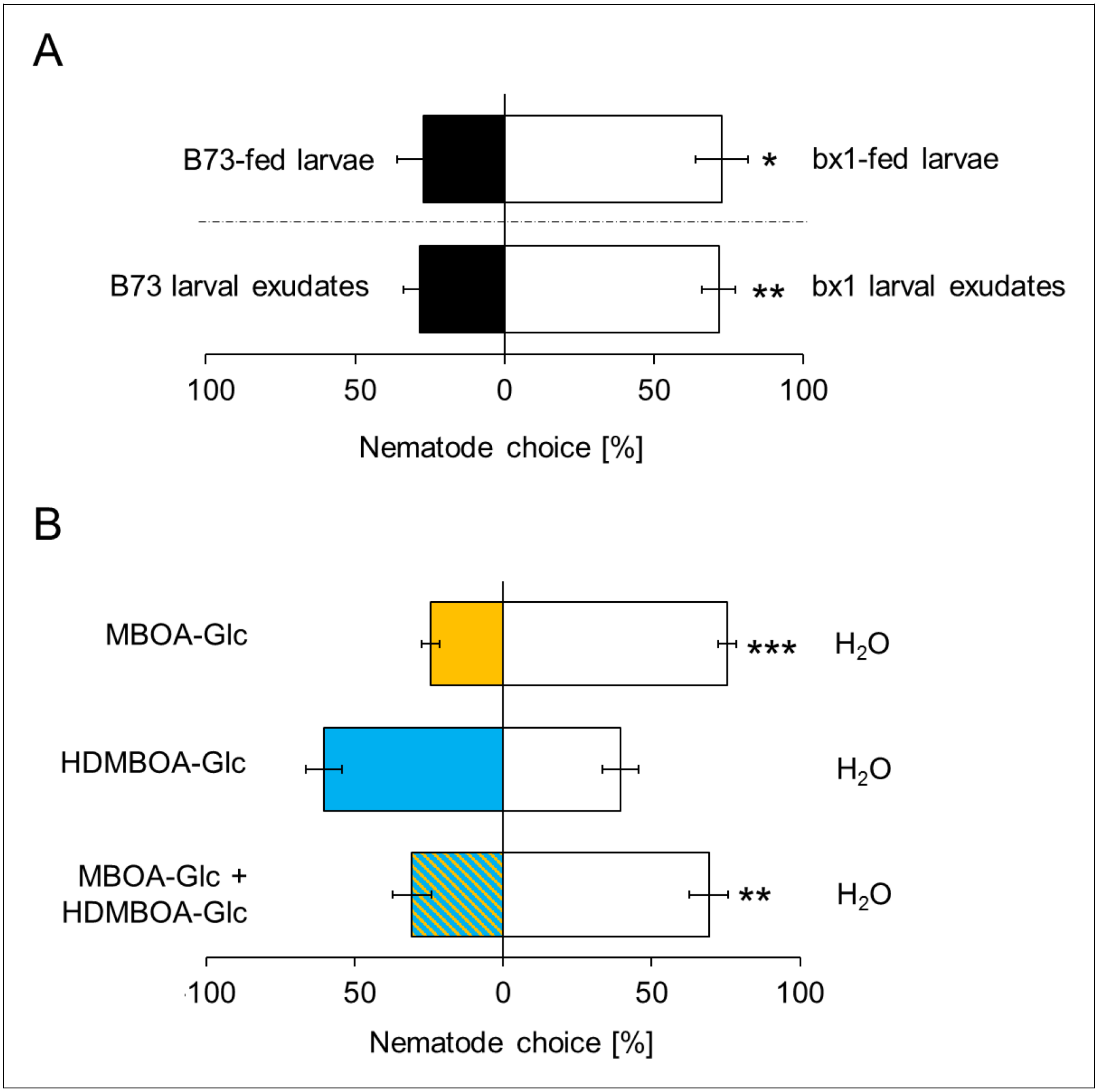

Figure 4. MBOA-Glc decreases the attractiveness of *Diabrotica virgifera* larvae. (**A**) Attraction of the entomopathogenic nematode (EPN) *Heterorhabditis bacteriophora to D. virgifera* larvae fed on wild-type (B73) and *bx1*-mutant (bx1:B73) (top) and aqueous surface extracts of larvae fed on wild 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***.

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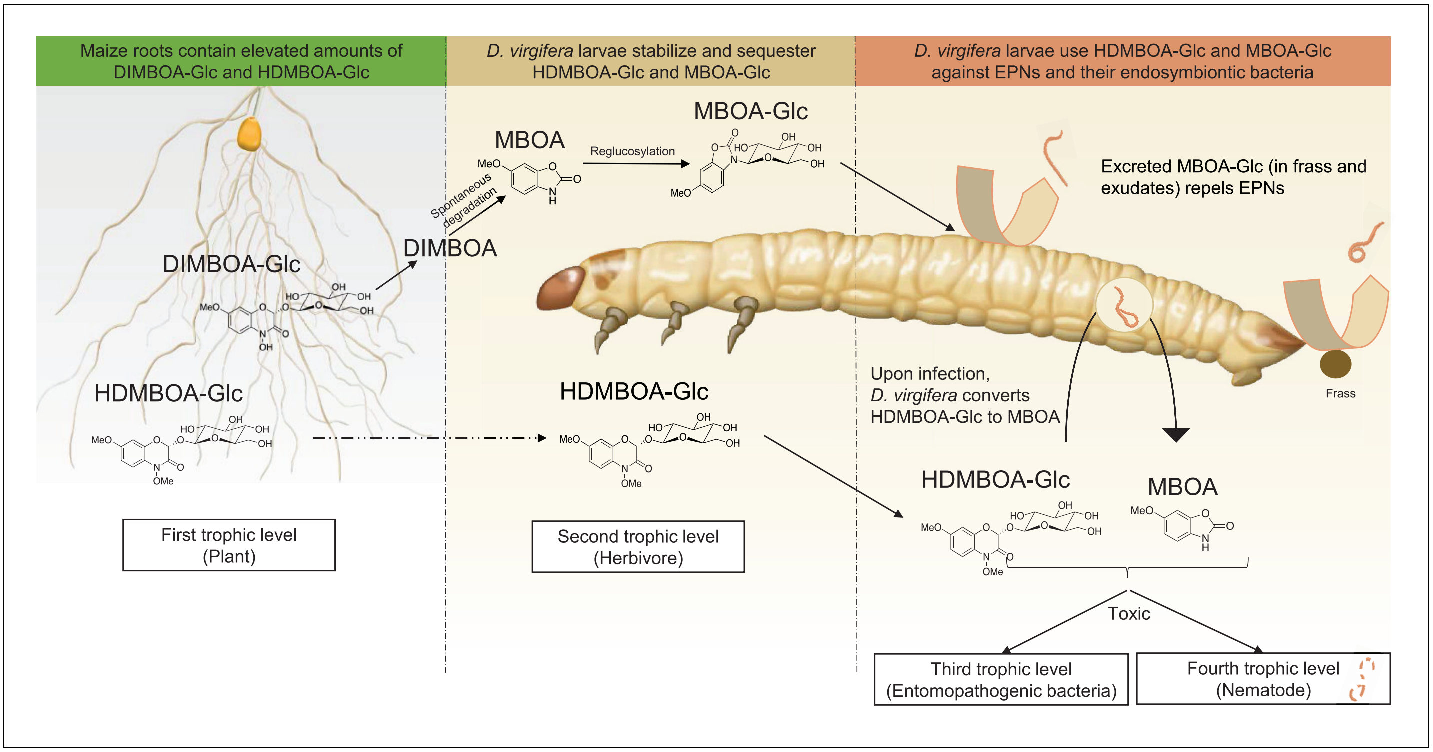

Figure 5. A model illustrating how BX sequestration and activation of plant toxins protects *Diabrotica virgifera* larvae from their enemies at multiple levels. MBOA-Glc, released in the frass and on the exoskeleton, repels infective juvenile entomopathogenic nematodes. Upon infection by nematodes and their symbiontic 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.

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