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Supplementary Information for

Plant defense resistance in natural enemies of a specialist insect herbivore

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Other supplementary materials for this manuscript include the following:

Dataset S1

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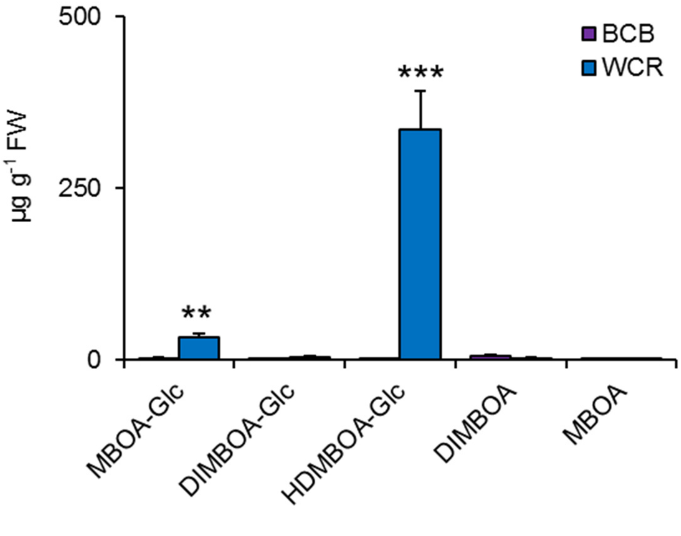

Fig. S1. Quantification of plant-derived benzoxazinoids in different nematode hosts. Absolute quantities of benzoxazinoids extracted from larvae of the western corn rootworm (WCR; blue) and the banded cucumber beetle (BCB; purple) are shown. Asterisks indicate significant differences between herbivore species (***P* <0.01; *** *P* <0.001).

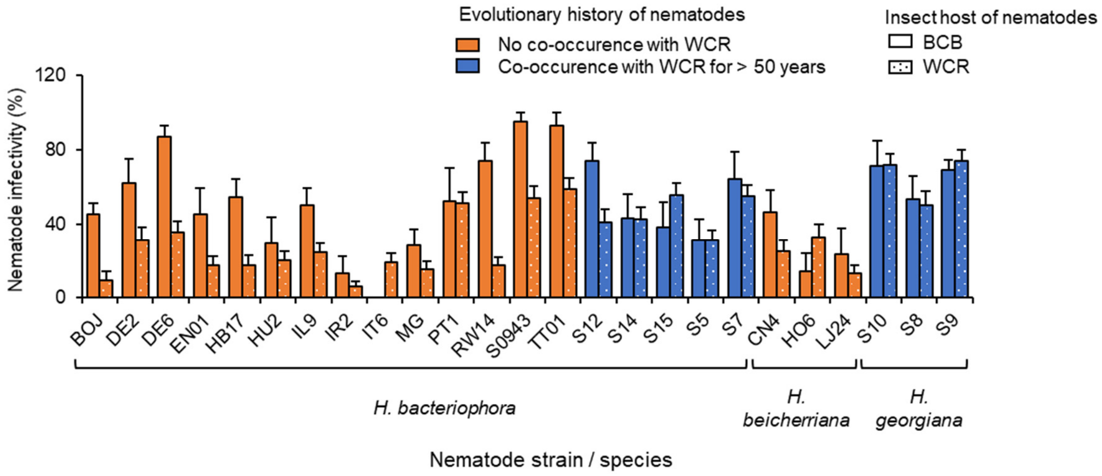

Fig. S2. Infectivity of individual nematode strains towards different herbivores. Infectivity of individual nematode strains from the primary range of the western corn rootworm (blue) and other parts of the world (orange) towards the western corn rootworm (WCR; plain filled bars) and the banded cucumber beetle (BCB; dotted bars) is shown.

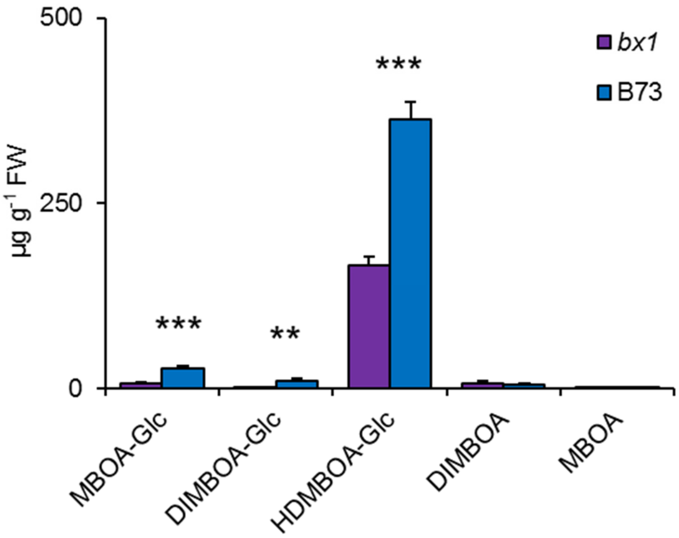

Fig. S3. Quantification of benzoxazinoids in western corn rootworm larvae fed on wild type and *bx1* mutant maize roots. Absolute quantities of benzoxazinoids extracted from larvae of the western corn rootworm fed on *bx1* mutant (*bx1*; purple) and wild type (WT; blue) maize roots are shown. Asterisks indicate significant differences between plant genotypes (***P* <0.01; *** *P* <0.001).

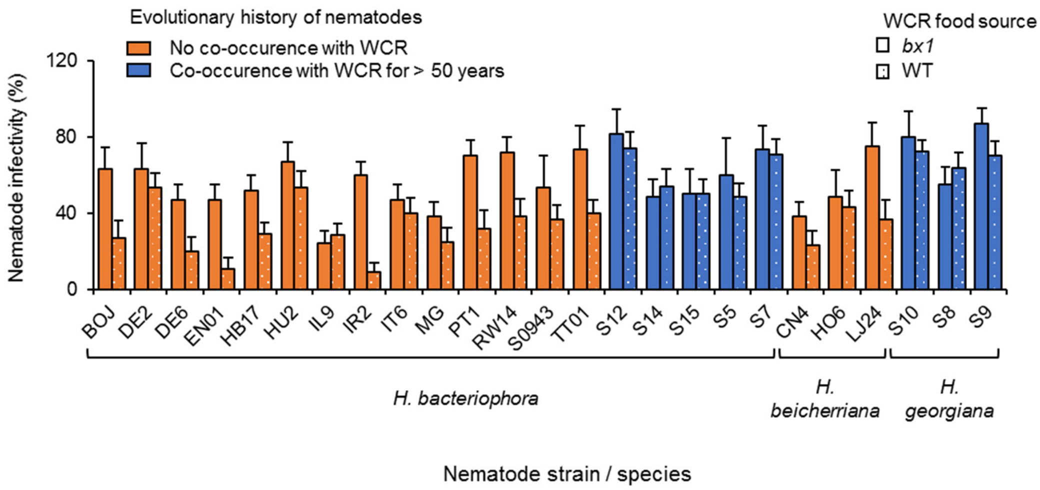

Fig. S4. Impact of herbivore-sequestered benzoxazinoids on the infectivity of individual nematode strains. Infectivity of individual nematode strains from the primary range of the western corn rootworm (WCR; blue) and other parts of the world (orange) towards WCR larvae fed on *bx1* mutant (*bx1*; plain filled bars) and wild type (WT; dotted bars) maize roots is shown.

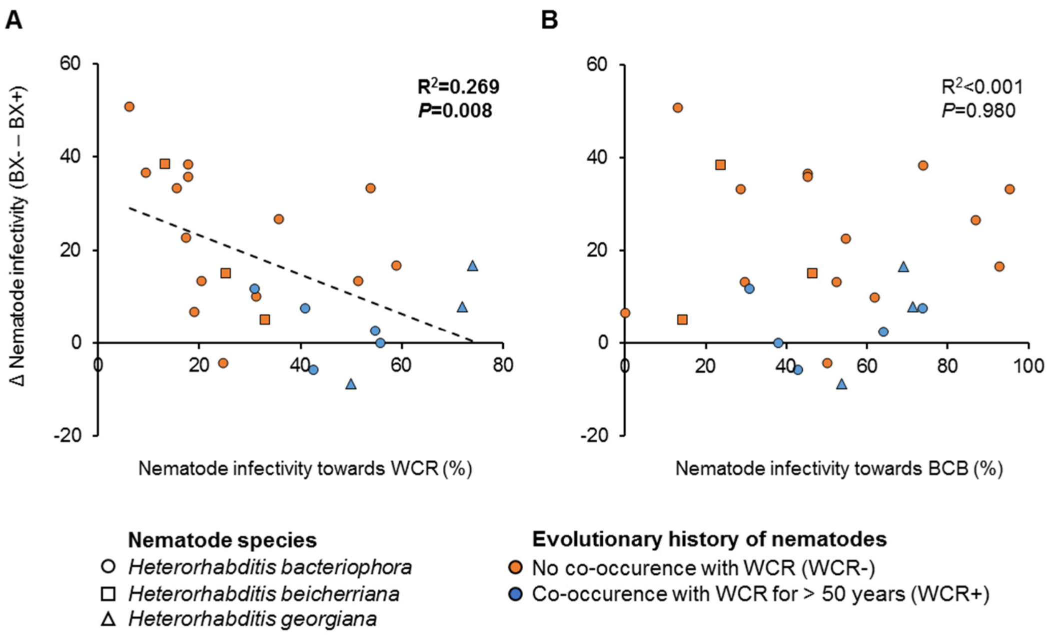

Fig. S5. Correlations between benzoxazinoid-dependent infectivity and infectivity towards different herbivores. Correlations are shown between the capacity of the different nematode strains to infect western corn rootworm (WCR) and banded cucumber beetle (BCB) larvae (Fig. S2) and their capacity to withstand sequestered benzoxazinoids (Fig. S4). Benzoxazinoid resistance is calculated by taking the difference in infectivity of the individual strains between *bx1* mutant (BX-) and wild type (BX+) maize root fed western corn rootworm larvae. Positive values correspond to higher infectiveness towards *bx1* mutant fed larvae. Significant correlations are indicated with dashed lines. R^2 and *P*-values from Pearson product-moment correlations are provided.

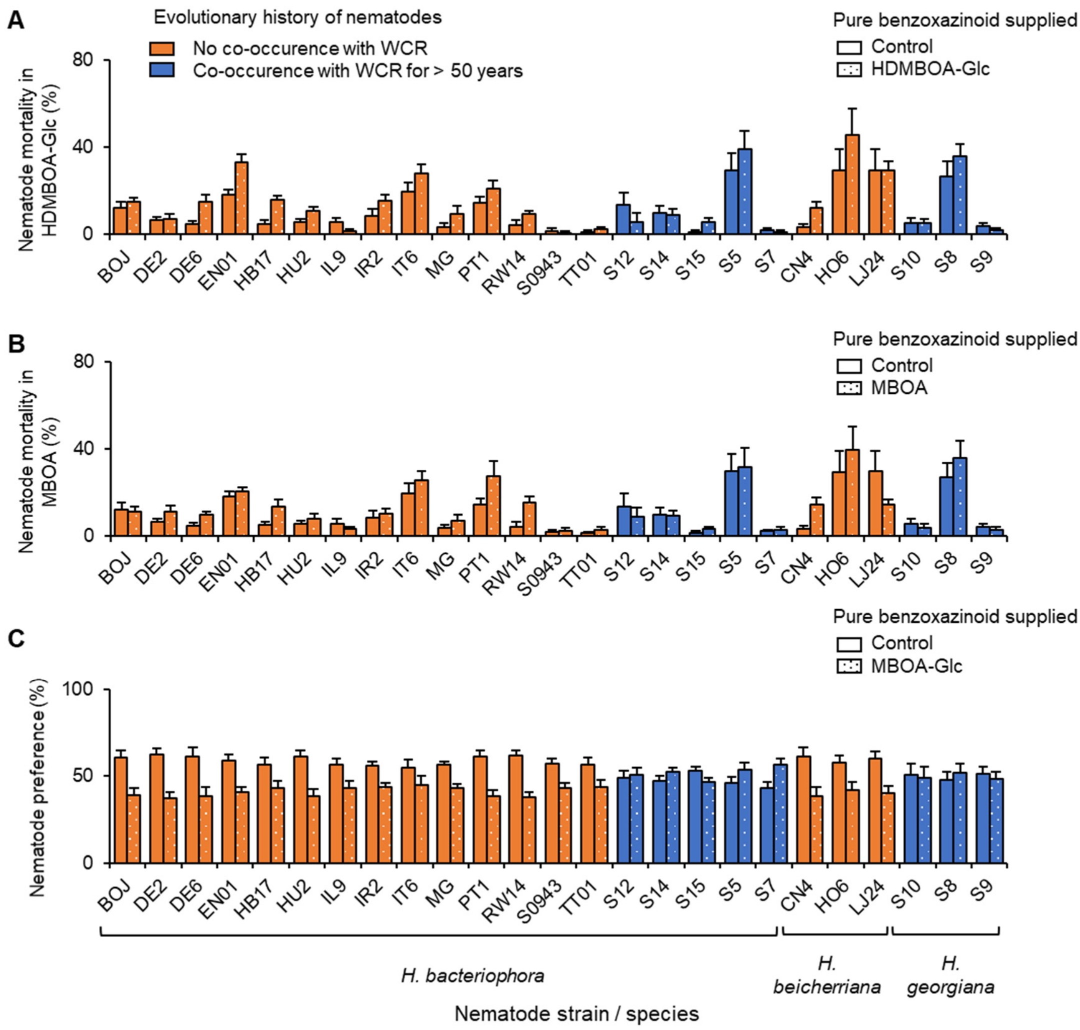

Fig. S6. Impact of pure benzoxazinoids on individual nematode strains. (A) Mortality of individual nematode strains from the primary range of the western corn rootworm (WCR; blue) and other parts of the world (orange) treated with water (plain filled bars) or 150 µg/mL HDMBOA-Glc (dotted bars) is shown. **(B)** Mortality of nematodes treated with water or 25 µg/mL MBOA. **(C)** Preference of nematodes for water or 3 µg/mL MBOA-Glc.

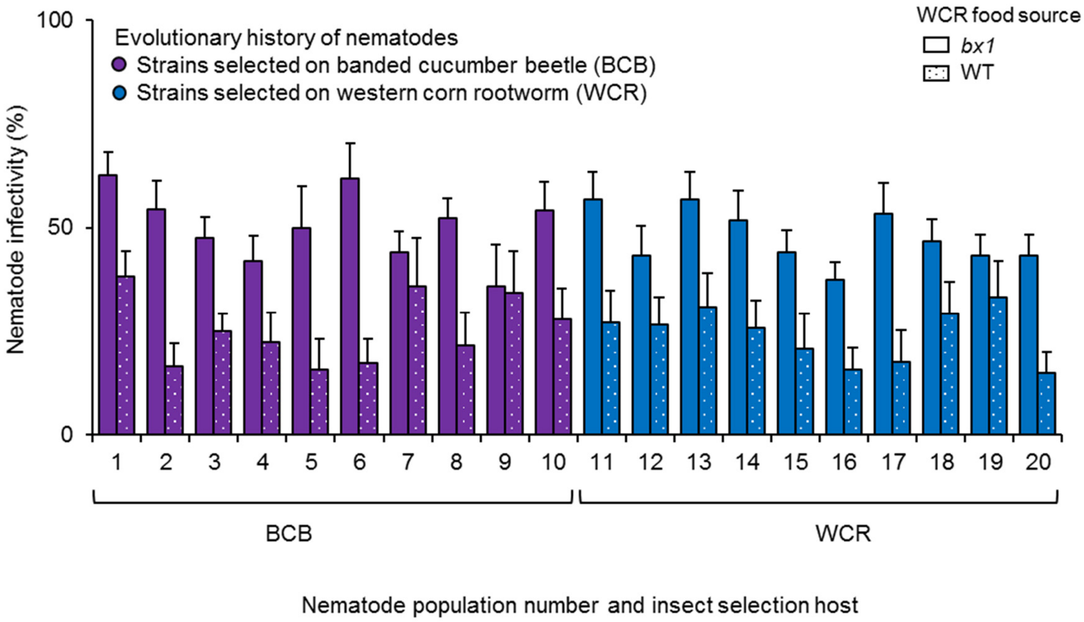

Fig. S7. Impact of herbivore-sequestered benzoxazinoids on nematode infectivity after one generation of artificial selection on different herbivores. Infectivity of individual nematode strains selected on the western corn rootworm (WCR; blue) or the banded cucumber beetle (BCB; purple) towards WCR larvae fed on *bx1* mutant (*bx1*; plain filled bars) and wild type (WT; dotted bars) maize roots is shown.

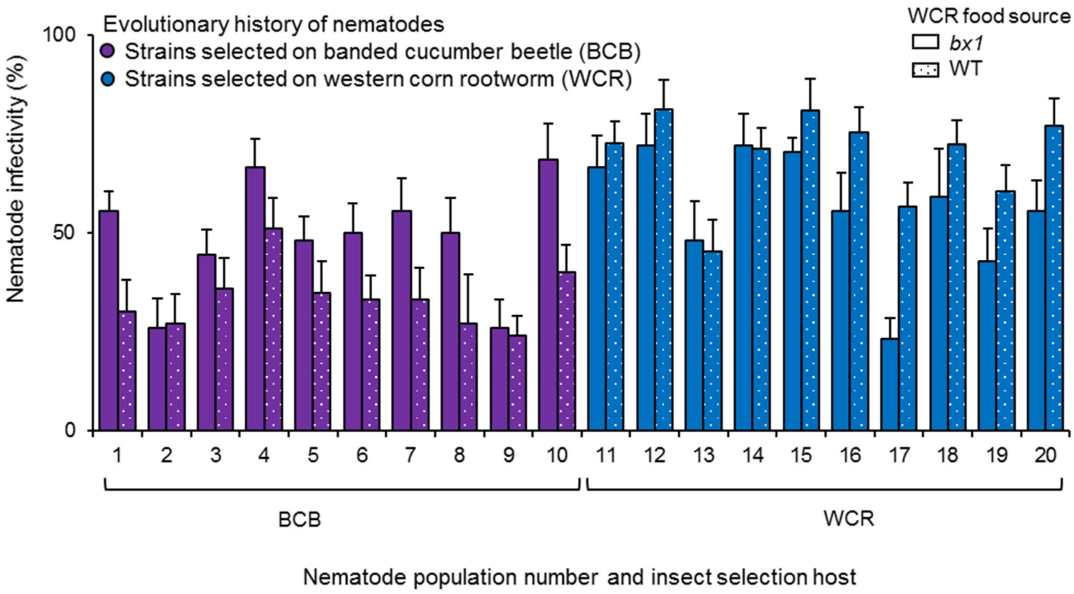

Fig. S8. Impact of herbivore-sequestered benzoxazinoids on nematode infectivity after five generations of artificial selection on different herbivores. Infectivity of individual nematode strains selected on the western corn rootworm (WCR; blue) or the banded cucumber beetle (BCB; purple) towards WCR larvae fed on *bx1* mutant (*bx1*; plain filled bars) and wild type (WT; dotted bars) maize roots is shown.

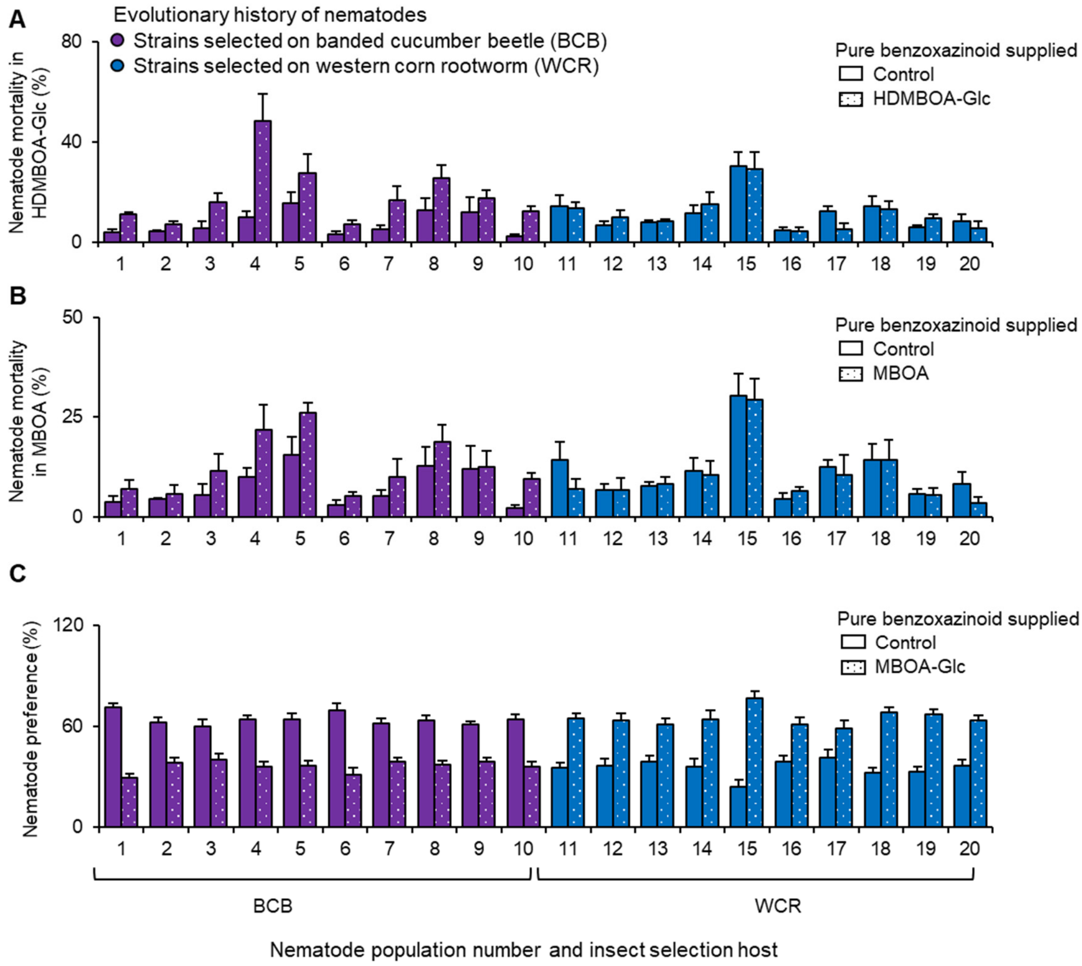

Fig. S9. Impact of pure benzoxazinoids on individual nematode populations after five generations of artificial selection. (A) Mortality of individual nematode strains after five generations of selection on the western corn rootworm (WCR; blue) or the banded cucumber beetle (BCB; purple) treated with water (plain filled bars) or 150 µg/mL HDMBOA-Glc (dotted bars) is shown. **(B)** Mortality of nematodes treated with water or 25 µg/mL MBOA. **(C)** Preference of nematodes for water or 3 µg/mL MBOA-Glc.

Table S1. Source and evolutionary histories of the different nematode strains

Strain	Abbreviation	Potential evolutionary history with WCR (years)	Nematode species	Country of origin	Location within country	Source
Boj (Hbz 90,2,24)	BOJ	0	*H. bacteriophora*	Iran	Bojnourd	Kamali Shokoofeh, 2014[1]
HU 2	HU2	0*		Hungary	N.a.	e-nema GmbH
IR 2	IR2	0		Iran	N.a.	e-nema GmbH
DE 2	DE2	0*		Germany	N.a.	e-nema GmbH
DE 6	DE6	0*		Germany	N.a.	e-nema GmbH
EN 01	EN01	0**		Commercial	N.a.	e-nema GmbH
IL 9	IL9	0		Australia	N.a.	e-nema GmbH
IT 6	IT6	0*		Italy	N.a.	e-nema GmbH
MG 618b	MG	0		Switzerland	Le Cerneux-Péquignot	Raquel Campos-Herrera
RW14-N-C4a	RW14	0		Rwanda	Nyamagabe	X. Yan, 2016[2]
M13e	TT01	0		Republic of Trinidad and Tobago	N.a.	P. Constant, 1998[3]
Hb 17	HB17	0		Turkey	Kirklareli	T.C. Ulu, 2014[4]
PT 1	PT1	0		Portugal	N.a.	e-nema GmbH
09-43	S0943	0		Turkey	Aydin	I. Kepenekci, 2013[5]
S12	S12	52***		USA	Minnesota	Own collection
S14	S14	62***		USA	Kansas	Own collection
S15	S15	62***		USA	Kansas	Own collection
S5P8	S5	52***		USA	Illinois	Own collection
S7	S7	52***		USA	Iowa	Own collection
CN 4	CN4	0	*H. beicherriana*	China	N.a.	e-nema GmbH
H06	HO6	0		China	Shandong	R.C. Han, 1996[6]
LJ-24	LJ24	0		China	Liaoning	J. Ma, 2013[7]

S10	S10	62		USA	South Dakota	Own collection
S8	S8	62	*H. georgiana*	USA	Nebraska	Own collection
S9	S9	62		USA	Nebraska	Own collection

N.a.: Not available
** Nematodes were collected prior to invasion of Europe by the western corn rootworm*
*** Artificially generated strain*
****Nematodes were isolated from maize fields within the primary range of the western corn rootworm*

Table S2. Numbers of individual biological replicates measured in the different experiments

Main figure	Supplementary figure	No.	Nematode strains	Treatment	Number of independent replicates per treatment/ nematode strain	Unit for replicate
Figure 1B	Figure S1			BCB/WCR	5	Five herbivores
Figure 1C	Figure S2		All strains	BCB	7*	Solo cup containing 3-5 individual herbivores
			LJ24	WCR	22	
			Other strains		24	
Figure 2A	Figure S3			BX- /BX+	5	Five herbivores
Figure 2B	Figure S4		All strains	BX-	5*	Solo cup containing 3-5 individual herbivores
				BX+	10	
Figure 2D	Figure S6	A	All strains	Control/HDMBOA-Glc	10	Flask containing 4000 nematodes
Figure 2E		B	All strains	Control/MBOA	10	
Figure 2F		C	Boj	MBOA-Glc	19	Petri dish containing 100 nematodes
			HU 2		18	
			Other strains		20	
Figure 3A	Figure S7		All lines	BX- /BX+	10	Solo cup containing 3-5 individual herbivores
Figure 3B	Figure S8		Line 17	BX-	10	
			Other Lines		9	
			Line 17	BX+	10	
			Line 1, 2, 6, 8, 9, 11, 13, 19		11	
			Other Lines		16	
Figure 3D	Figure S9	A	All lines	Control/HDMBOA-Glc	8	Flask containing 4000 nematodes
Figure 3E		B	All lines	Control/MBOA	8	
Figure 3F		C	Line 12, 15, 17, 20	MBOA-Glc	19	Petri dish containing 100 nematodes
			Other Lines		20	

** Lower number of replicates are due to the lower availability of BCB and BX- WCR larvae at the time of experiment.*

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