Supplementary Materials

SpitWorm, an herbivorous robot: Mechanical leaf wounding with simultaneous application of salivary components

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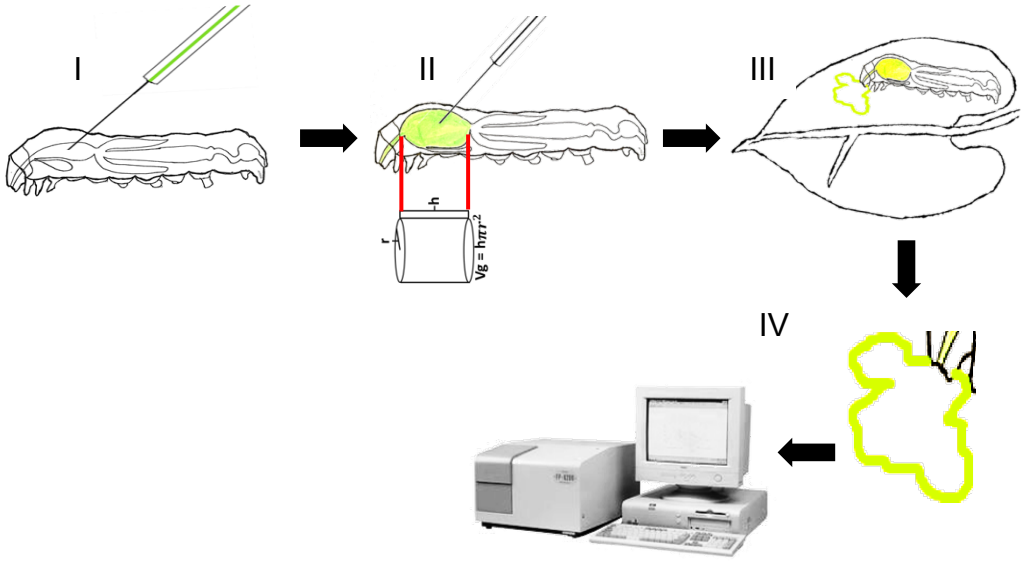


Figure S1. Workflow for determination of OS amount left at the leaf wounding edges. (I) *S. littoralis* larva injected with fluorescent dye into the foregut; (II) *S. littoralis* larva foregut dissected and measured as a cylinder; (III) fluorescent dye solution injected larva fed on *P. lunatus* leaf; (IV) fluorescence dye signal at the wounding area of the leaf being quantified.

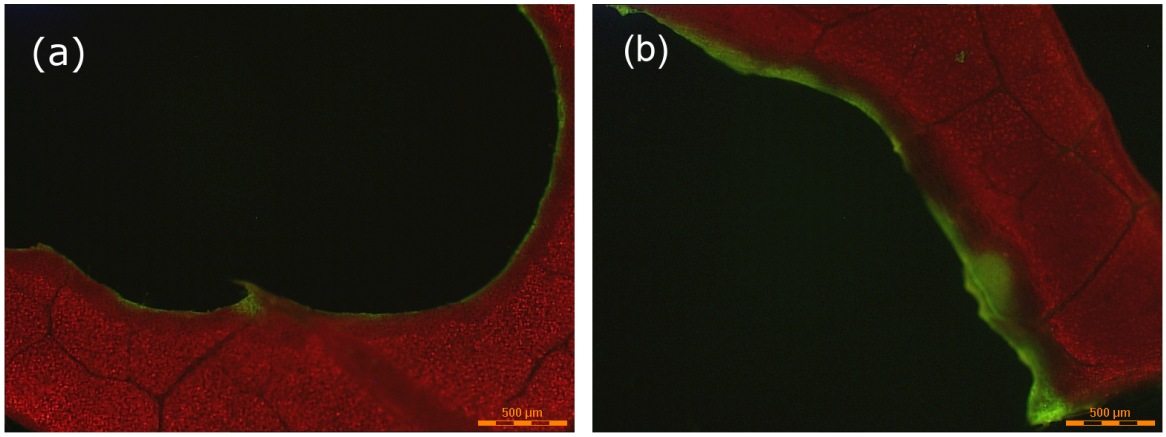


Figure S2. Comparison of fluorescence signals left in plant wounded sites by insects injected with fluorescent dye. (a) Leaf wounded by a *S. littoralis* larva with 1 µL injection; (b) wounded by a larva with 5 µL injection of a solution of Lucifer Yellow in water (1 mg·mL^{-1}).

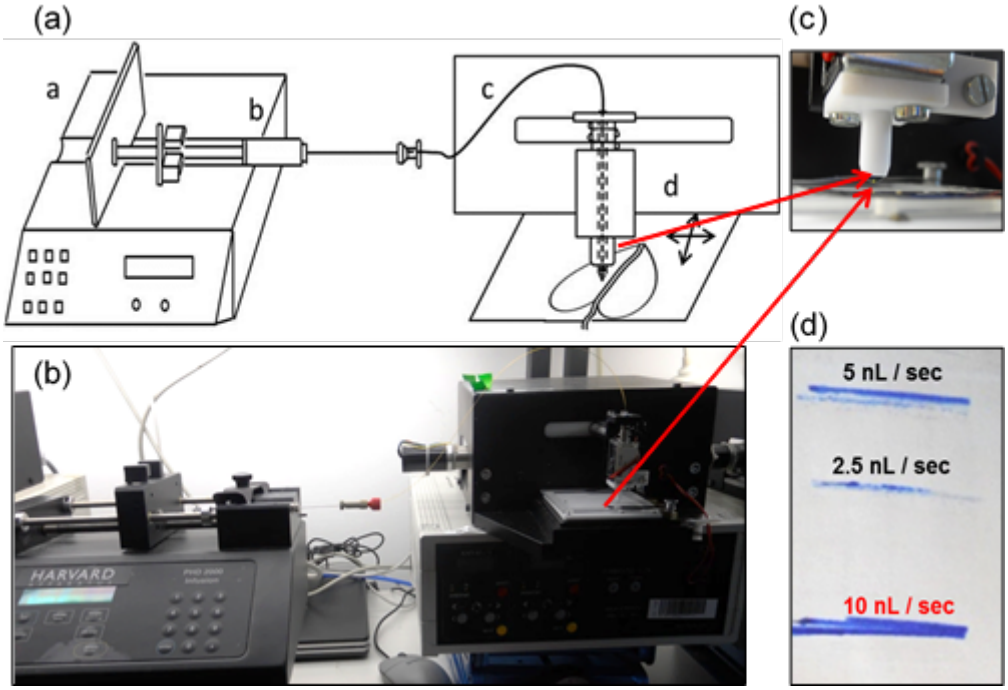


Figure S3. SpitWorm set-up and flow rate optimization. (a) Schematic sketch of SpitWorm: a; Syringe pump to control the delivery rate; b; 100 µL syringe; c; fused silica capillary connecting the syringe to MecWorm through the hollow needle which has a little hole at the tip. d; MecWorm, a system for controlled mimicking the feeding behavior of biting insects. (b) Picture of SpitWorm. (c) An enlarged picture of the 'tooth' of SpitWorm, with an ink droplet at the tip. (d) Ink trails left by SpitWorm at different fluid delivery rates.

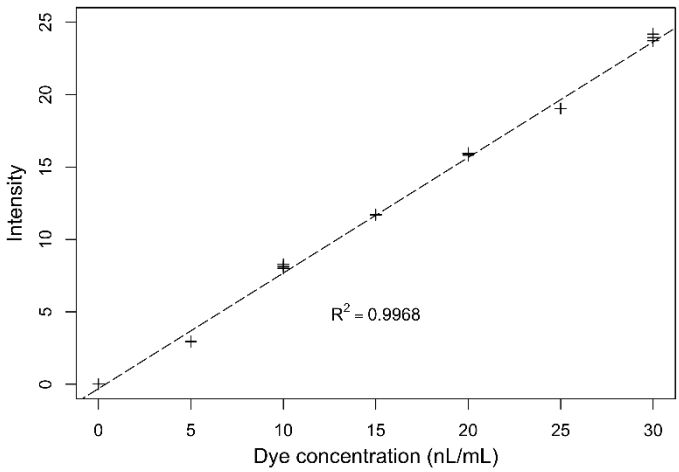
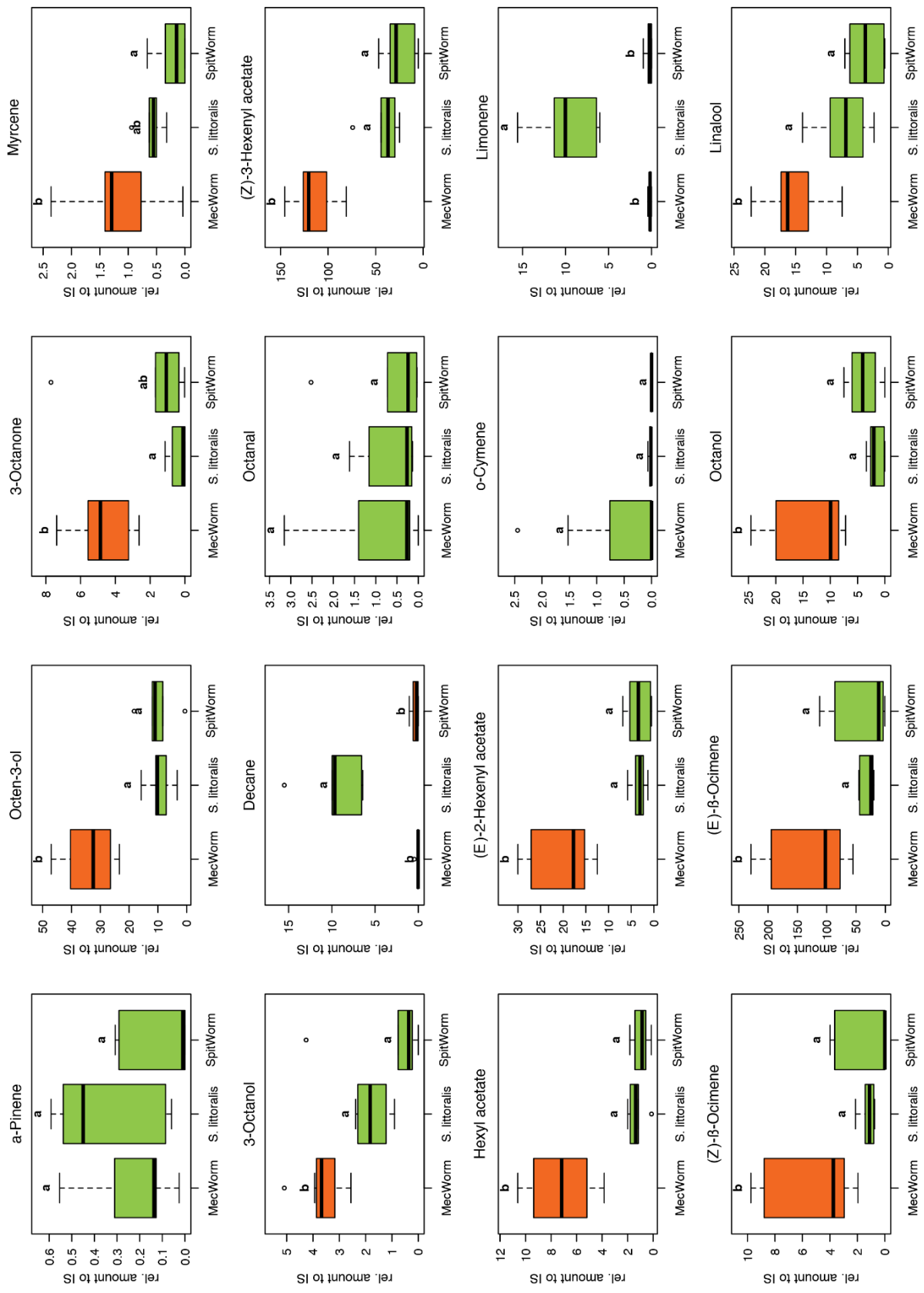


Figure S1. Standard curve. Fluorescent signal intensity of different dilutions (n = 3) of Lucifer Yellow solution (1 mg·mL^{-1}).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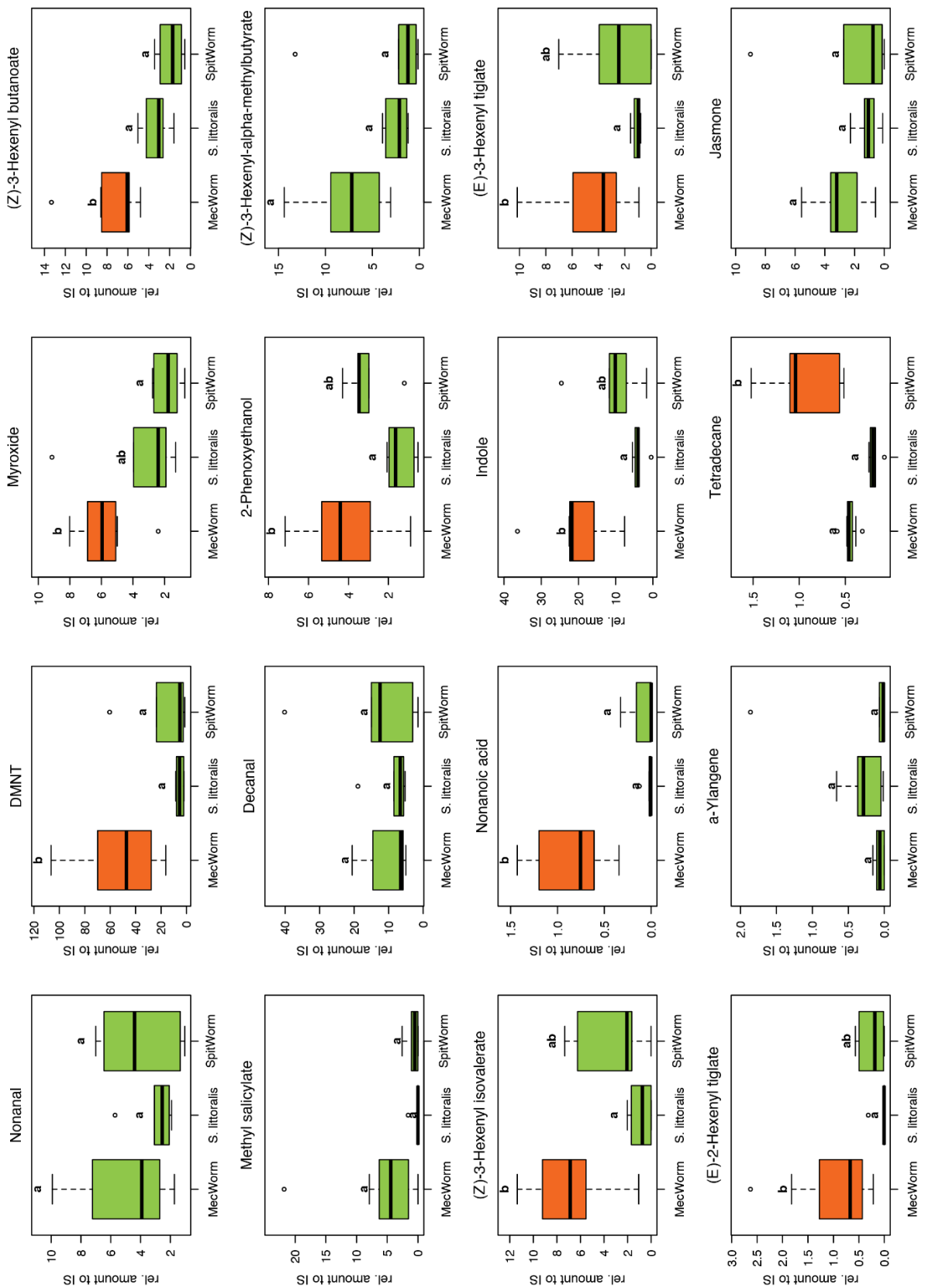
Octen-3-ol
Decane
3-Octanone
Myrcene
3-Octanol
Hexyl acetate
(E)-2-Hexenyl acetate
Octanal
o-Cymene
(Z)-3-Hexenyl acetate
Limonene
(Z)-β-Ocimene
(E)-β-Ocimene
Octanol
Linalool
rel. amount to IS
MecWorm
S. littoralis
SpitWorm

continued …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DMNT
Myroxide
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Methyl salicylate
Decanal
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(Z)-3-Hexenyl-alpha-methylbutyrate
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Nonanoic acid
Indole
(E)-3-Hexenyl tiglate
(E)-2-Hexenyl tiglate
a-Ylangene
Tetradecane
Jasmone
rel. amount to IS
MecWorm
S. littoralis
SpitWorm

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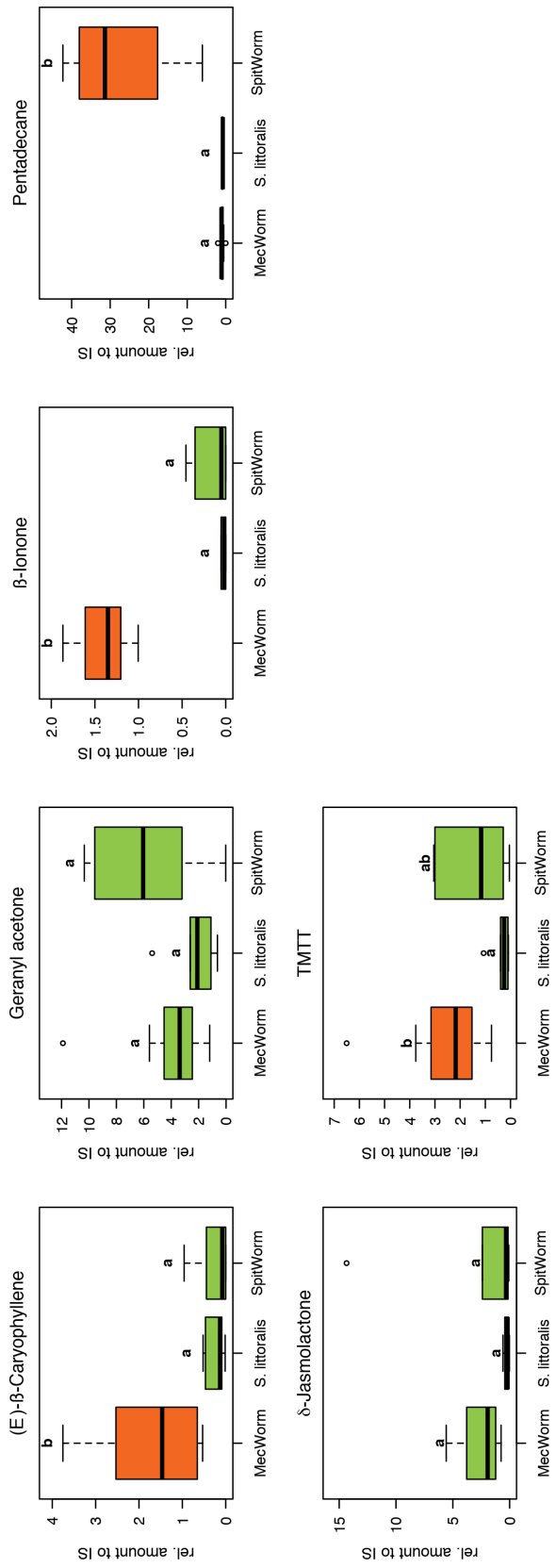

Figure S5. Comparison of relative amounts of headspace volatiles upon different treatments. Three different treatments on lima bean leaves (*S. littoralis larvae*, n = 6; MecWorm, n = 7; SpitWorm, n= 6). One-way ANOVA, post hoc test: Tukey's HSD, treatments with identical letters showed no significant difference, equal colors indicate no significant difference to SpitWorm treatment.

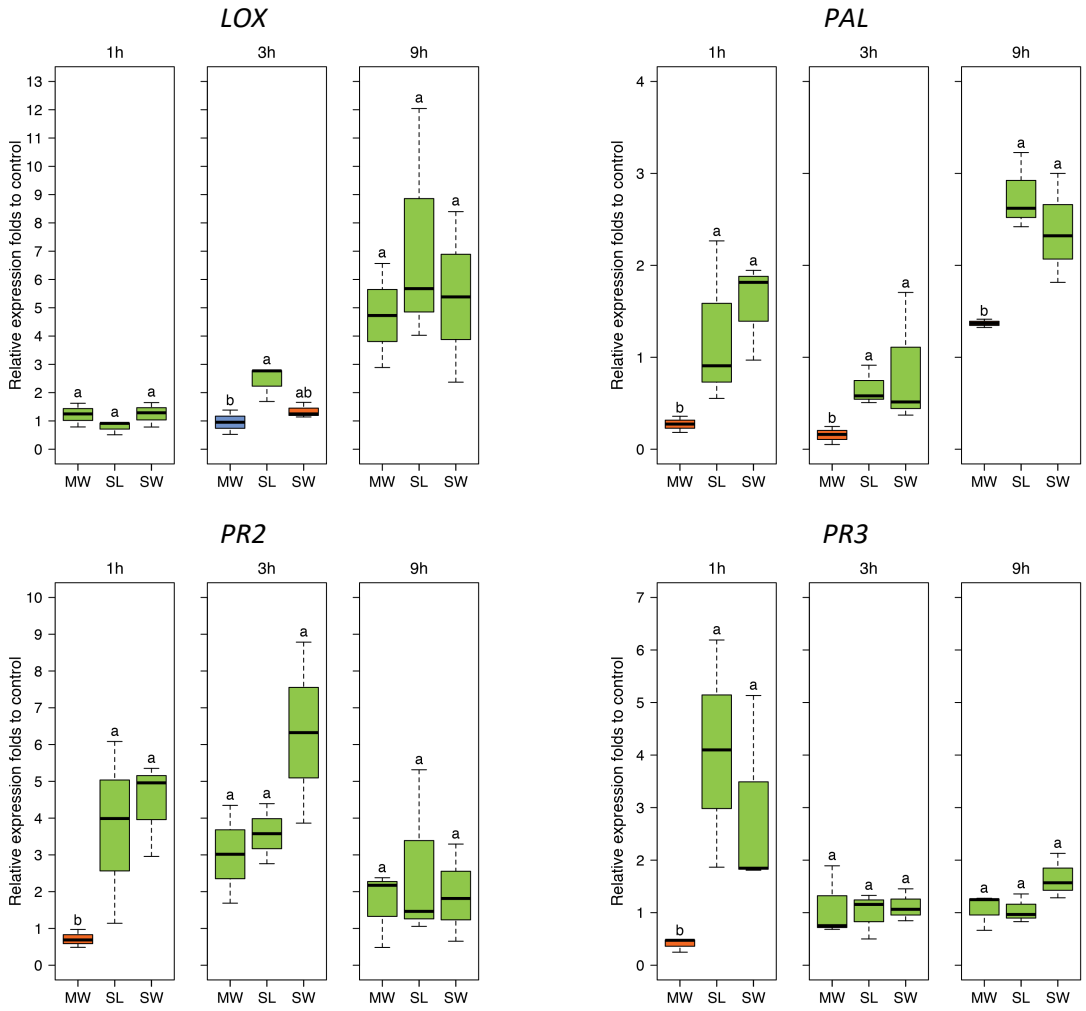

Figure S6. Expression of four JA responsive genes (*LOX3*, *PAL*, *PR2*, and *PR3*). Lima beans treated for 1 h, 3 h and 9 h with MecWorm (MW), *S. littoralis* (SL), and SpitWorm (SW; 10 times diluted OS, delivery speed of 10 nL·s^{-1}); n = 3 for each treatment, log2 transformed, one-way ANOVA, post-hoc test: Fisher's LSD, treatments with identical letters are not significantly different.

Table S1. Dimensions of larval foreguts. Lengths (l) and diameters (d) of dissected foreguts were measured. Foregut volume (Vg) was calculated by taking the shape of the foregut as a cylinder.

n	l (mm)	d (mm)	Vg (mm^3)
1	3	3	21.2
2	5	4	62.8
3	4.3	4	54.0
4	5	4	62.8
5	4	3.8	44.2
mean	4.3	3.8	49.0
sd	0.8	0.4	17.3