Supporting Information

Article title: *The 2-C-methyl-D-erythritol-2,4-cyclodiphosphate metabolite modulates defense responses against aphid in Arabidopsis*

Authors: Nawaporn Onkokesung[1†], Michael Reichelt[2], Louwrance P. Wright[2], Michael A. Phillips[3], Jonathan Gershenzon,[2] and Marcel Dicke[1*]

The following Supporting Information is available for this article:

Table S1 Specific primers used for quantitative real-time PCR.

Gene	Sequence (5′→3′)
HDS-Forward	CAGAATGCGTAACACTAAGAC
HDS-Reverse	GAGAACCACCTACATATCCG
MYB51-Forward	CTACAAGTGTTTCCGTTGACTCTGAA
MYB51-Reverse	ACGAAATTATCGCAGTACATTAGAGGA
CYP81F2-Forward	TATTGTCCGCATGGTCACAGG
CYB81F2-Reverse	CCACTGTTGTCATTGATGTCCG
WRKY70-Forward	ACCCGTTAAGGGTAAAAGAGGA
WRKY70-Reverse	CTTGGGTTCGAGCTCAACCT
AOS-Forward	TCCACCCAAAAACCGTACGA
AOS-Reverse	TGAAGAACTCTTCAGCTCCTTG
HPL-Forward	GCTGAGAACGGTTGGAAAAC
HPL-Reverse	TCCGGCGATTAAGAGAGAAG
ICS1-Forward	CACTAGATTCTCCCGCAAGAAG
ICS1-Reverse	TGGTCAATTGGAACCTGTAACC
VSP2-Forward	TCAGTGACCGTTGGAAGTTGTG
VSP2-Reverse	GTTCGAACCATTAGGCTTCAATATG
PR1-Forward	CACTACACTCAAGTTGTTTGGA
PR1-Reverse	TAGTATGGCTTCTCGTTCACAT
PAD3-Forward	CTGATCAGAAACCCAAGAGTGA
PAD3-Reverse	GTTTTCGCAGGAACATCGTAG
PAD4-Forward	AACAGAGATATAAAGACTGGCGGGC
PAD4-Reverse	ACACACTCCTCAGGCACTTTAACTC
TPS11-Forward	TGGTTCTCGTGCAGATTAC
TPS11-Reverse	TCCGAGATCGCGTAATAAG
EF-Forward	TGAGCACGCTCTTCTTGCTTTCA
EF-Reverse	GGTGGTGGCATCCATCTTGTTACA

Figure S1. Time course of relative transcript expression of *HDS* in response to feeding by *Pieris brassicae* caterpillars on (A) WT and (B) 35S:HDS (*hds3)* plants. The graphs represent average expression ± SE (n = 5) at each time point. The expression was compared among time point of each plant type by one-way ANOVA followed by Tukey's honestly significant difference (HSD) posthoc test). Different letters above bars indicate significant differences among time points ($P \leq 0.05$).

Figure S2. Accumulation of an intermediate metabolite and final metabolites of the MEP pathway in undamaged and aphid-damaged leaf tissue of WT, *hds3,* and 35S:HDS (*hds3*) plants. The effects of a high accumulation of MEcPP on other metabolites in MEP pathway were determined by quantification via HPLC/MS of (A) the intermediate metabolite 1-deoxy-D-xylulose 5-phosphate DXP and (B) the final metabolites isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in undamaged (control) and aphid-damaged tissue from WT, *hds3* and 35S:HDS (h*ds3*) plants. Values represent average ± SE (n = 5) of DXP and IPP+DMAPP levels in leaf tissue of each plant type. Data were compared within the genotype by Student's *t*-test, different letters indicate significant difference ($P \leq 0.05$).

Figure S3. Accumulation of aliphatic glucosinolates in undamaged and aphid damaged leaf tissue of WT, *hds3*, or 35S:HDS(*hds3*) plants. The aliphatic glucosinolates were quantified by HPLC/MS analysis of leaf tissue of WT, *hds3* and 35S:HDS (h*ds3*) plants. Values represent means ± SE (n = 5) for undamaged (A) and aphid-infested (7d) (B) plants. Data were compared among WT, *hds3* and 35S:HDS (*hds3*) plants by one-way ANOVA followed by Tukey's honestly significant difference (HSD) posthoc test. Different letters indicate significant differences between plant genotypes ($P \leq 0.05$).

Abbreviations: 3MSOP: 3-methylsulphinylpropyl glucosinolate; 4MTB: 4-methylthiobutyl glucosinolate; 4MSOB: 4-methylsulphinylbutyl glucosinolate; 7MSOH: 7-methylsulphinylheptyl glucosinolate; 8MSOO: 8-methylsulphinyloctyl glucosinolate.

Figure S4. Accumulation of aliphatic, indolic and total glucosinolate levels in undamaged and aphid-damaged leaf tissue of WT, *hds3,* or 35S:HDS(*hds3*) plants. Impact of a high

accumulation of MEcPP on glucosinolate accumulation in response to cabbage aphid feeding. Aliphatic glucosinolates and indolic glucosinolates were quantified by HPLC/MS for undamaged or aphid-damaged leaf tissue of WT, *hds3* and 35S:HDS (h*ds3*) plants. The sum of (A) aliphatic glucosinolates, (B) indolic glucosinolates and (C) the total levels of (aliphatic+indolic) glucosinolates are presented. The data were compared among WT, *hds3* and 35S:HDS (*hds3*) plants by one-way ANOVA followed by Tukey's honestly significant difference (HSD) posthoc test. Different letters indicate significant differences within each plant genotype ($P \leq 0.05$).

Figure S5. Relative transcript expression of a marker gene of SA biosynthesis and a marker gene of SA response in WT leaves in response to an exogenous application of synthetic MEcPP or ME. Synthetic MEcPP or ME was exogenously applied on a fully expanded leaf of wild type plants to determine the effects of MEcPP on the SA signaling pathway. The relative transcript expression of SA biosynthetic gene (*ICS1*) and a marker gene of SA responsive gene (*PR1*) was quantified at 2h after exogenous application of MEcPP or ME. The average transcript expression ± SE (n = 4) is presented. Data were compared among treatments by one-way ANOVA followed by Tukey's honestly significant difference (HSD) posthoc test. Different letters indicate significant differences among treatments ($P \leq 0.05$).

Figure S6. Kinetics of the relative transcript expression of *hydroperoxide lyase* (*HPL*) in response to feeding by the aphid *B. brassicae* in leaf tissue of WT, *hds3*, or 35S:HDS(*hds3*) plants. Average transcript expression levels ± SE (n = 5) are presented for different time points Data were compared among plant genotypes at each time point by one-way ANOVA followed by Tukey's honestly significant difference (HSD) posthoc test. Different letters indicate significant differences among plant genotype ($P \leq 0.05$)

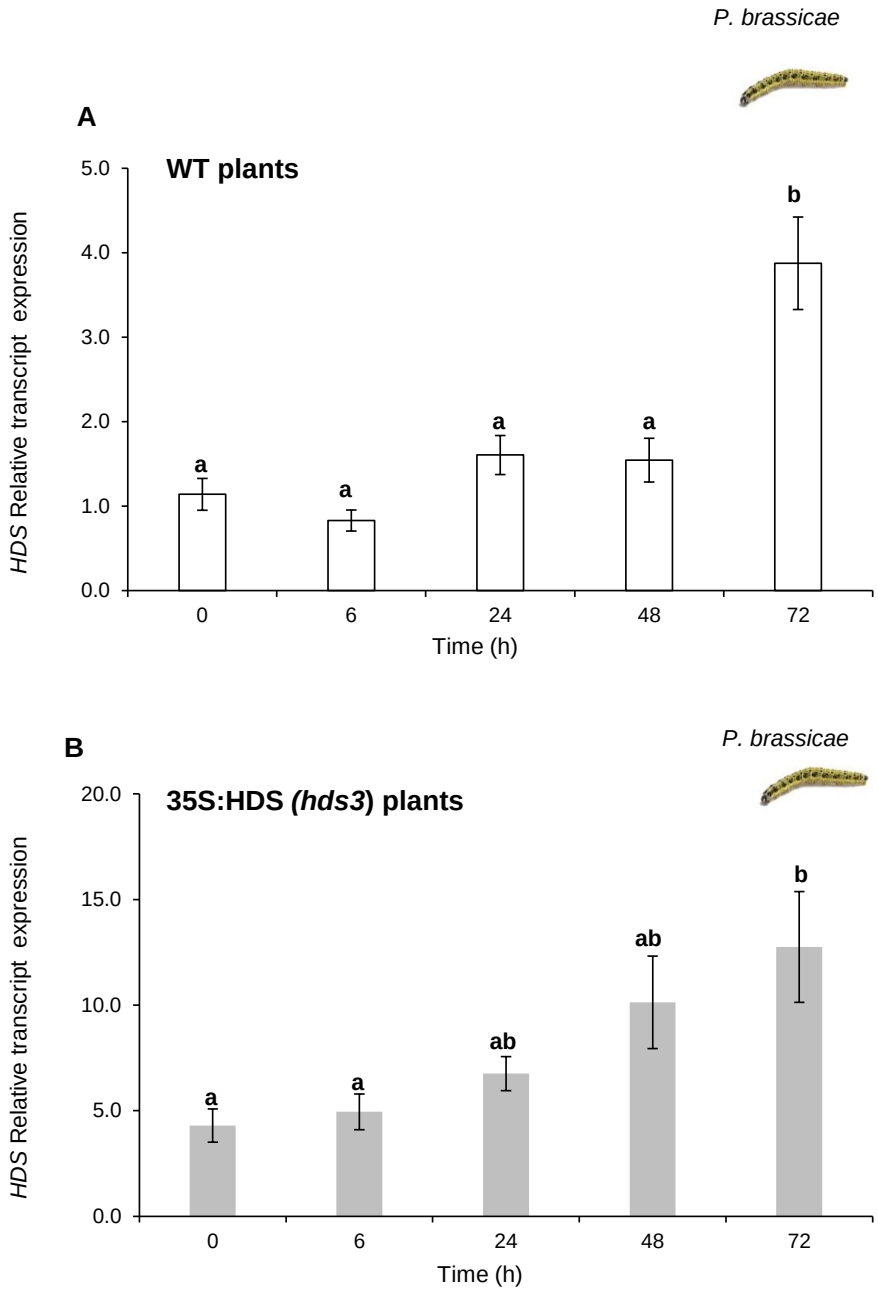
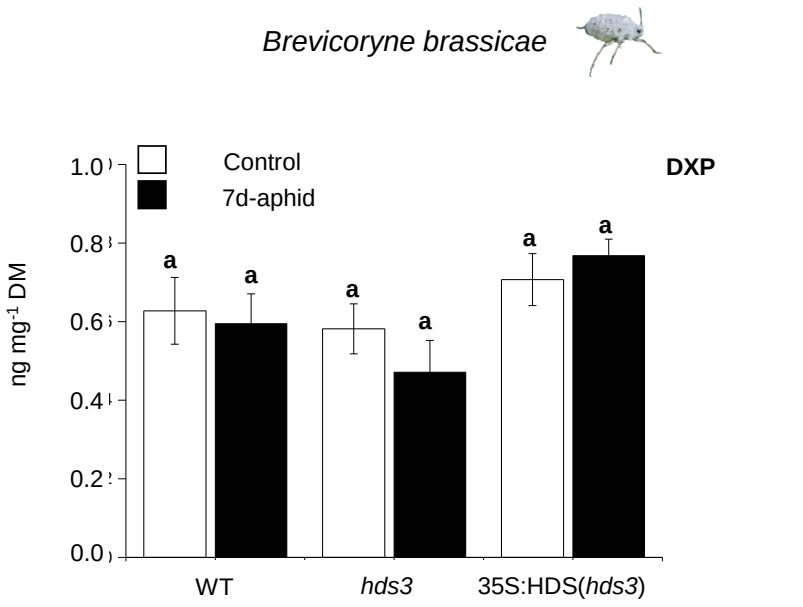

P. brassicae
A
WT plants
HDS Relative transcript expression
5.0
4.0
3.0
2.0
1.0
0.0
a
a
a
a
b
0
6
24
48
72
Time (h)
P. brassicae
B
35S:HDS (hds3) plants
HDS Relative transcript expression
20.0
15.0
10.0
5.0
0.0
a
a
ab
ab
b
0
6
24
48
72
Time (h)

Figure S1


Brevicoryne brassicae
Control
7d-aphid
DXP
ng mg-1 DM
1.0
0.8
0.6
0.4
0.2
0.0
a
a
a
a
a
a
WT
hds3
35S:HDS(hds3)

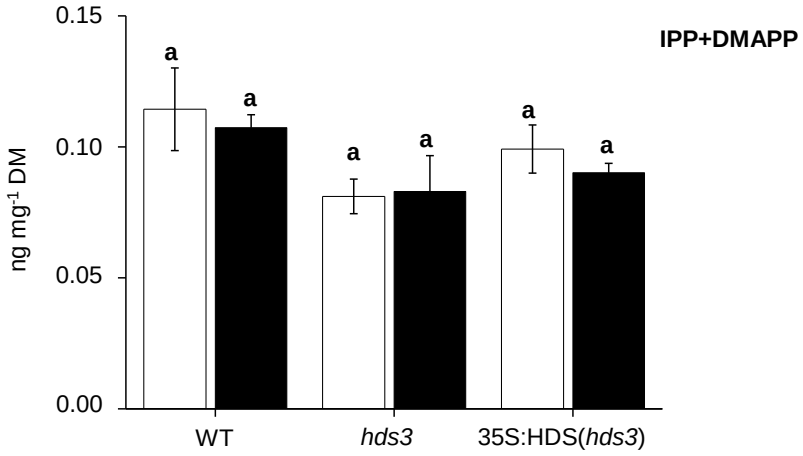

IPP+DMAPP
ng mg-1 DM
0.15
0.10
0.05
0.00
a
a
a
a
a
a
WT
hds3
35S:HDS(hds3)

Figure S2

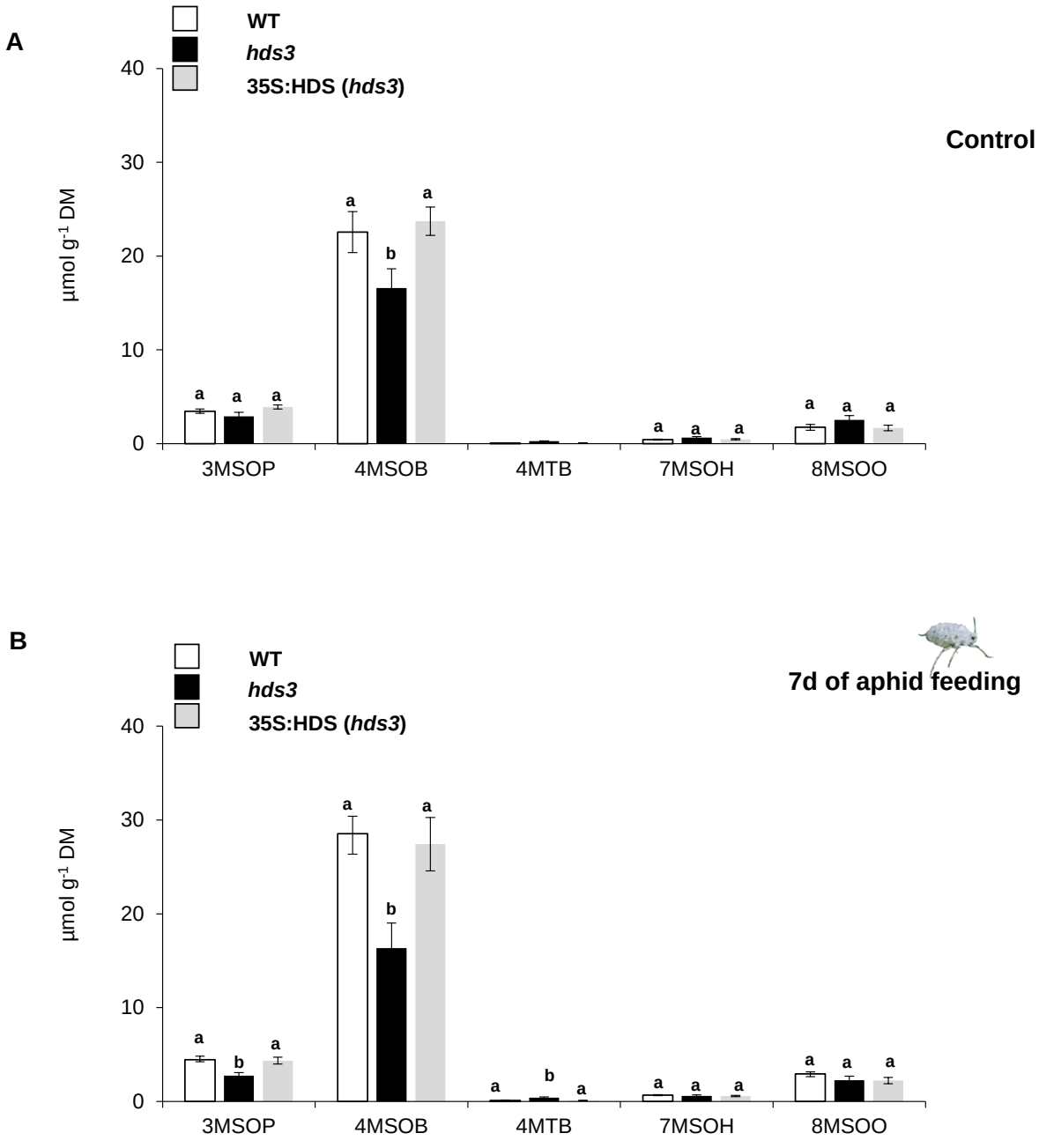

A
WT
hds3
35S:HDS (hds3)
Control
µmol g-1 DM
40
30
20
10
0
a a a
a b a
a a a
a a a
a a a
3MSOP
4MSOB
4MTB
7MSOH
8MSOO
B
WT
hds3
35S:HDS (hds3)
7d of aphid feeding
µmol g-1 DM
40
30
20
10
0
a b a
a b a
a b a
a a a
a a a
3MSOP
4MSOB
4MTB
7MSOH
8MSOO

Figure S3

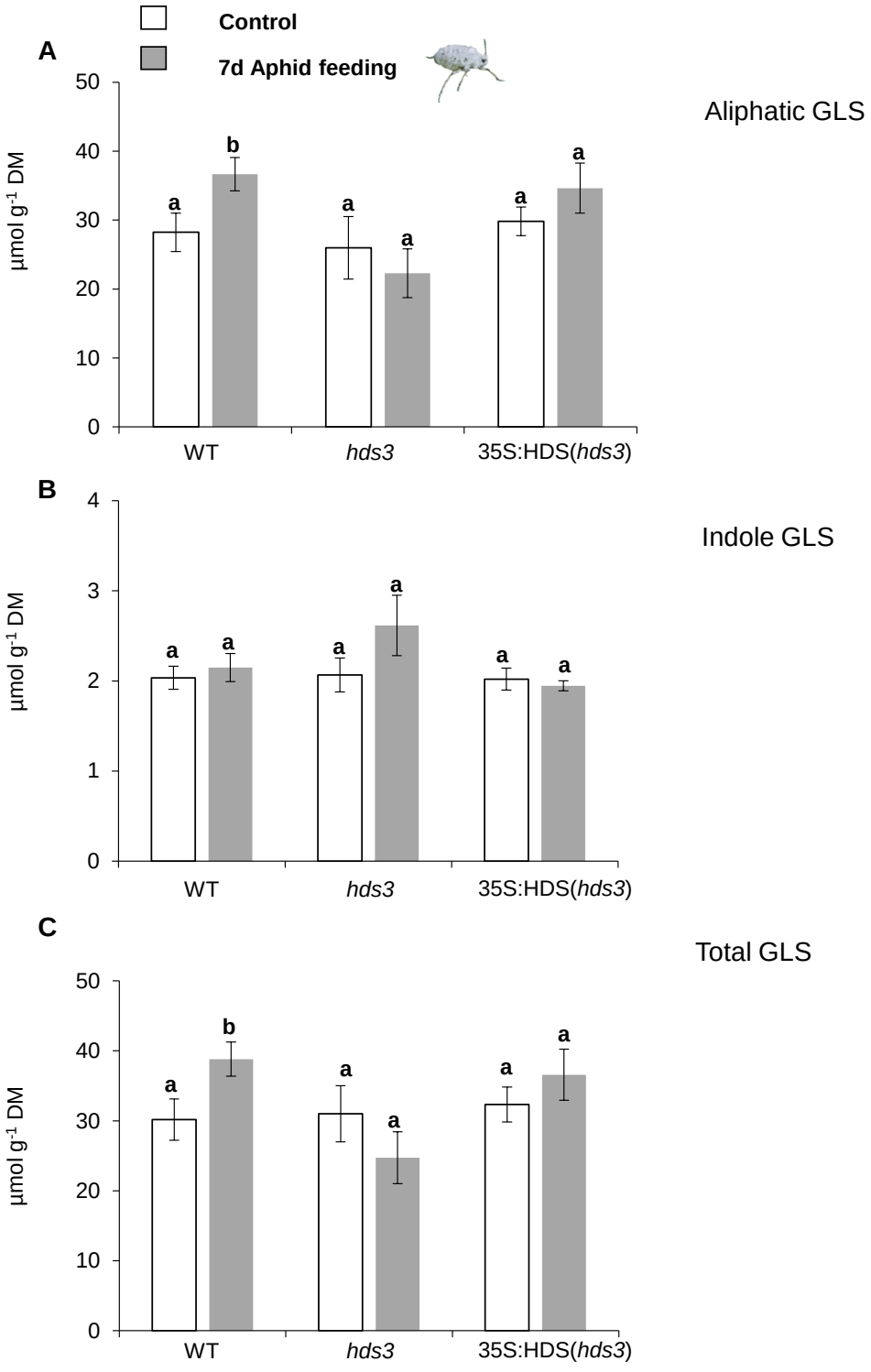

A
Control
7d Aphid feeding
Aliphatic GLS
µmol g-1 DM
50
40
30
20
10
0
a
b
a
a
a
a
WT
hds3
35S:HDS(hds3)
B
Indole GLS
µmol g-1 DM
4
3
2
1
0
a
a
a
a
a
a
WT
hds3
35S:HDS(hds3)
C
Total GLS
µmol g-1 DM
50
40
30
20
10
0
a
b
a
a
a
a
WT
hds3
35S:HDS(hds3)

Figure S4

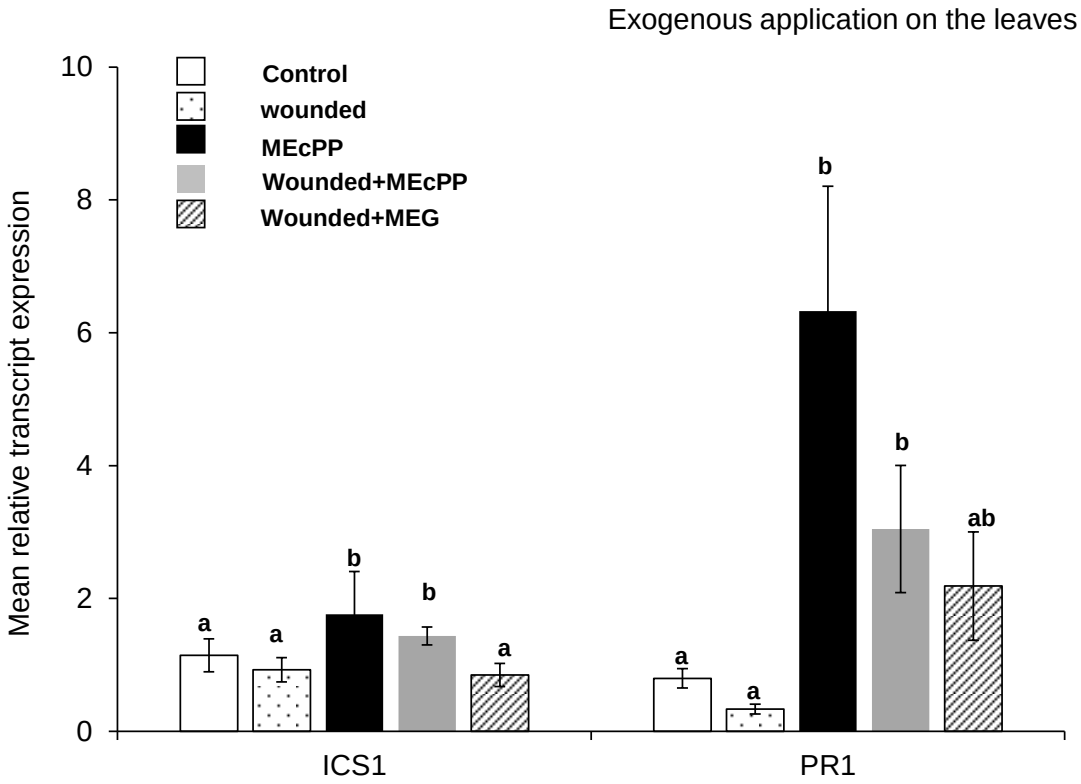

Exogenous application on the leaves
Mean relative transcript expression
10
8
6
4
2
0
Control
wounded
MEcPP
Wounded+MEcPP
Wounded+MEG
a
a
b
b
a
a
a
b
b
ab
ICS1
PR1

Figure S5

Brevicoryne brassicae

HPL

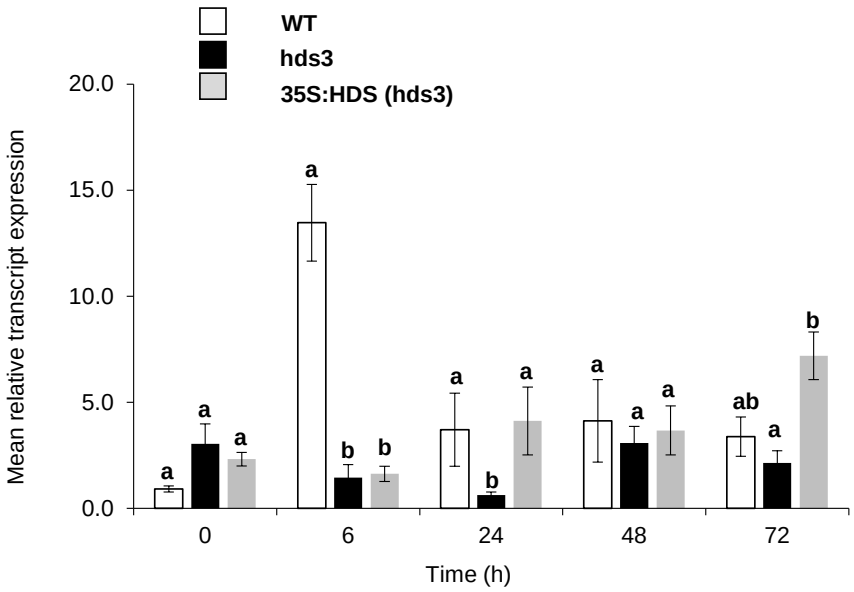


Figure S6