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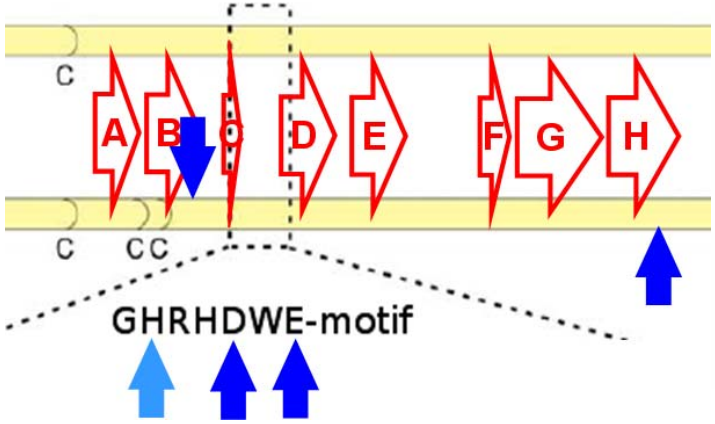
Nep1-like proteins (NLPs) of *Verticillium longisporum*

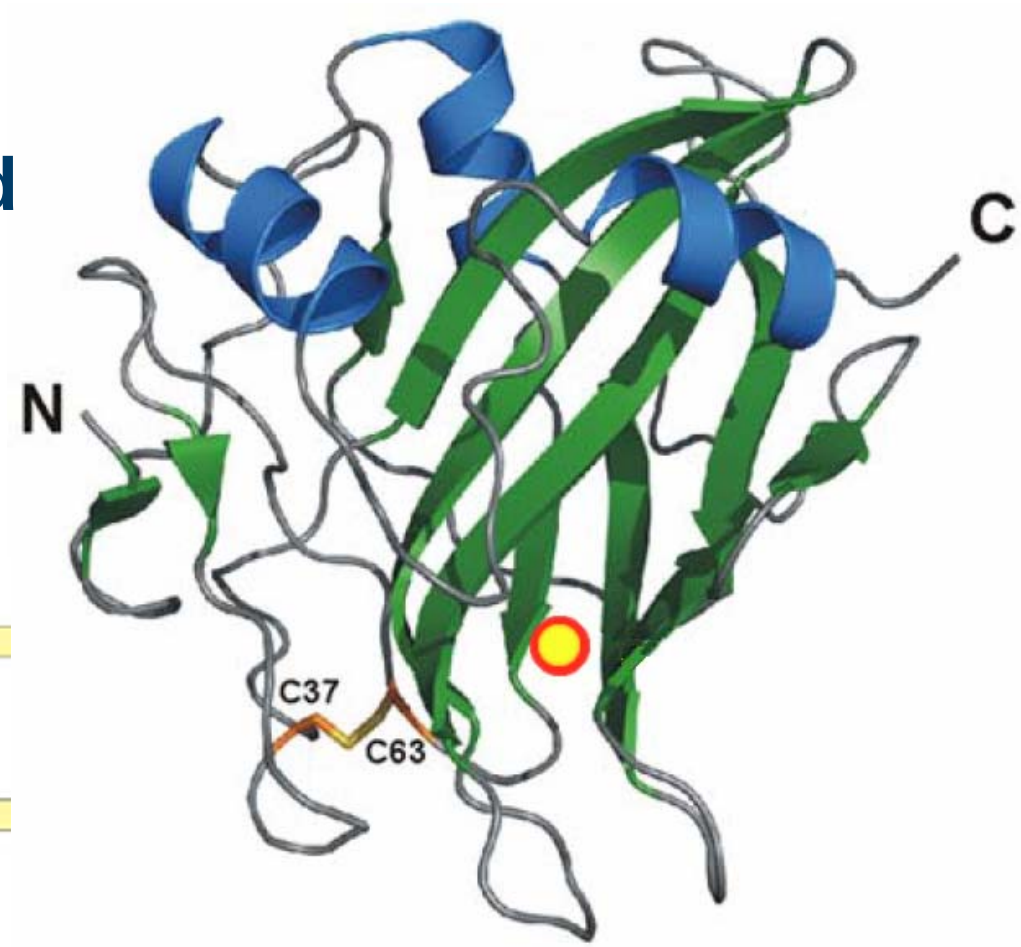
Petr Karlovsky

Presented at the meeting of DFG Research Unit FOR 546 in February 2011. Results contributed by Malte Beinhoff, Ruth Pilot, Mona Quambusch, Richard Splivallo, Arne Weiberg, Haiquan Xu, and Haidi Yin

Nep-1 like proteins (NLPs)

NLP_{Pya}
3D-structure solved





Cechin et al. (2008) BMC Plant Biol 8:50

Nep-1 like proteins

Biological activity

- Phytotoxicity
- Plasma membrane permeabilization
- Activation of defense responses

All effect are limited to dicotyledons.

Nep-1 like proteins

Biological function ?

- **<u>Virulence factor ?</u>**
  - Indirect evidence: transcription control, heterologous expression
  - No evidence from gene inactivation
- Attachment to host surface ??
- Suppression of microbial competitors ??
- Unknown enzymatic activity ??

NLP genes of *Verticillium longisporum*

VINEP	ORF (nt)	aa sequence	Mol. weight (Da)	intron	aa sequence identity (Vd)	aa sequence identity (Vaa)
1	760	233	25,986	207-267	229/233 (98%)	224/233 (96%)
2	831	256	27,361	321-380	251/256 (98%)	234/267 (88%)
3	834	277	31,040	-	262/283 (93%)	269/277 (97%)
4	927	308	35,327	-	292/308 (95%)	283/296 (96%)
5	970	264	29,420	92-149 577-631 829-890	264/264 (100%)	185/295 (63%)

Malte, Haiquan, Arne

NLP proteins of *Verticillium longisporum*

```
VlNEP-2   LDFDTDGCYNTAAIDPDGNINPGKG-ATGTPQGDCRDPPQLENSNVYSRRRCNNGVCAIM
VlNEP-3   LDFDTDSCYNVPAIGPNGDLAIGMYPFEWPPQAGCRNEEMLDRGNVYSRQRCNNGYCVIF
VlNEP-4   VDIDTDSCYSVPAMTMDGTASEGLS--PSDDVGPCRPRSALDRSNVYVRGRCNRGWCAFV
VlNEP-1   LHIAHG-CQPYSAVNTRGEVNAGLQ-DSGTTAGGCKETS---KGQTYARSMTLNGQFGIM
VlNEP-5   PEAGQTDGVKTDGVDSDLCFSSQNGGCADPGPWNGVNSPGNPFPVYYTVRQCNDNEWRVA
           .         .:                                 *      .   .

VlNEP-2   YEYYFEKDQSVSGSF-AGGHRHDWEN-VVVFARG----DTIVRVAPSCHGGYGG------
VlNEP-3   YAYYFQKDTATP----IDGHRHDWEH-IAVWVRQSD--SFVTHVAVSQHKGYDIRENS--
VlNEP-4   YAYYFQMDWAWSWPVSSYNHRHDWEH-VVVWAKE----GKVRGVSVSQHGGYENRVAEDQ
VlNEP-1   YAWYWPKDQPADGNL-ASGHRHDWEN-VVIWFNSNN--ANQAGILRGAASGHGDYK----
VlNEP-5   YSIYYKKD---------SGHKNDWENSIVIWNGDGAGGWKRSGTLLGWHSGWDYIAWG--
          *  *:  *          .*::***:  :.::                .   *
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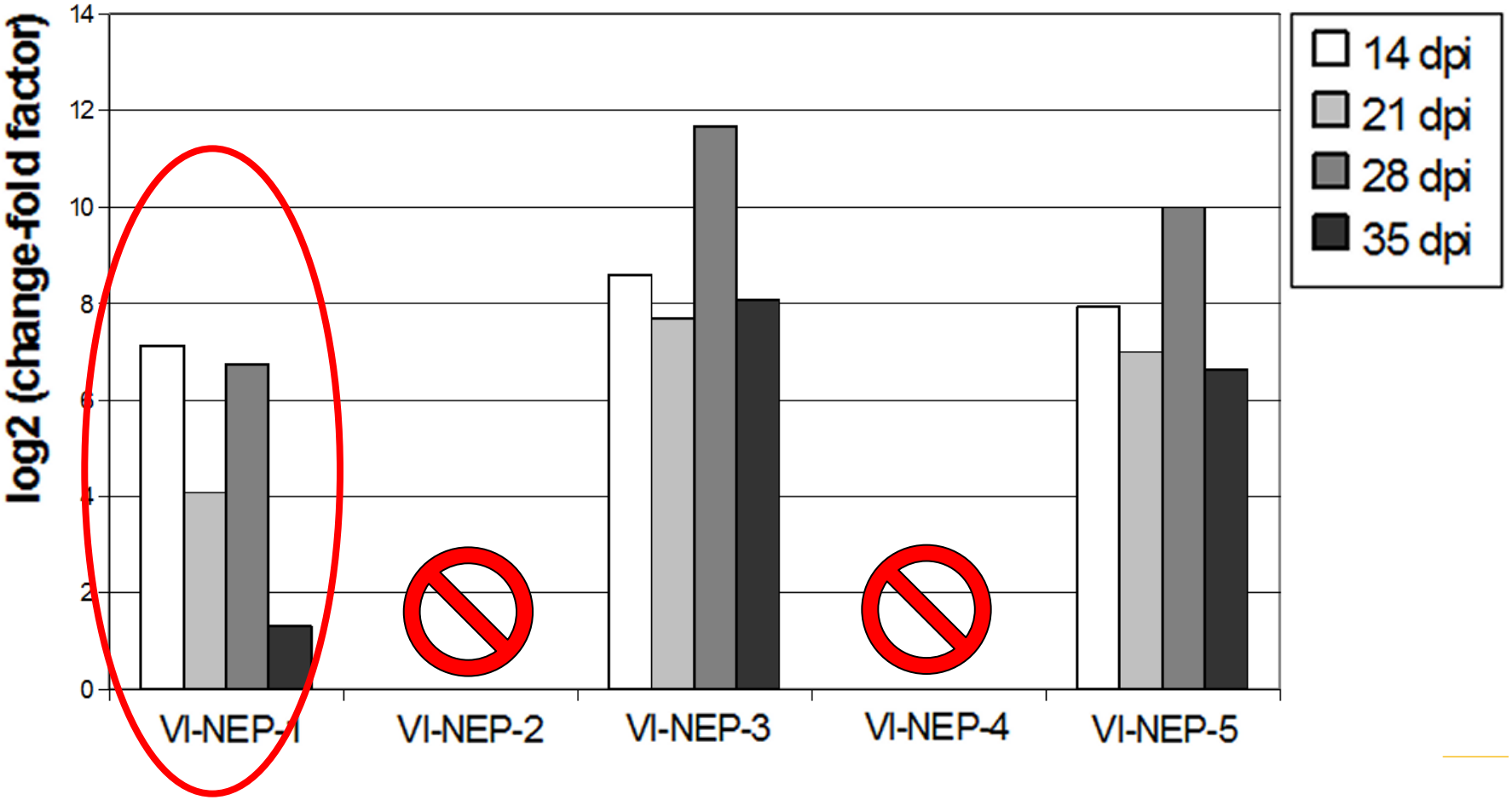
Involved in Mg^{++} binding

Malte, Haiquan, Arne

Similarity to VlNEPs to other NLPs

In planta expression of VlNEPs



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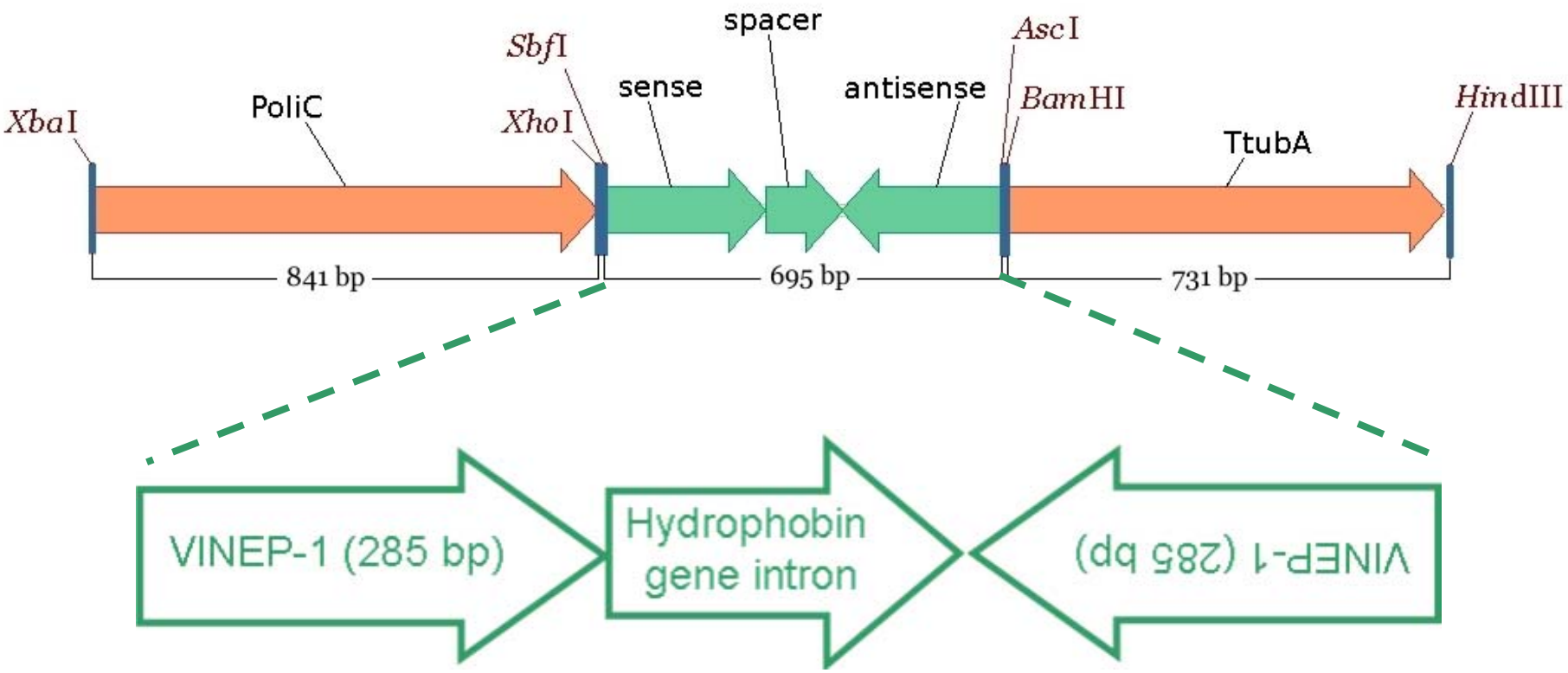
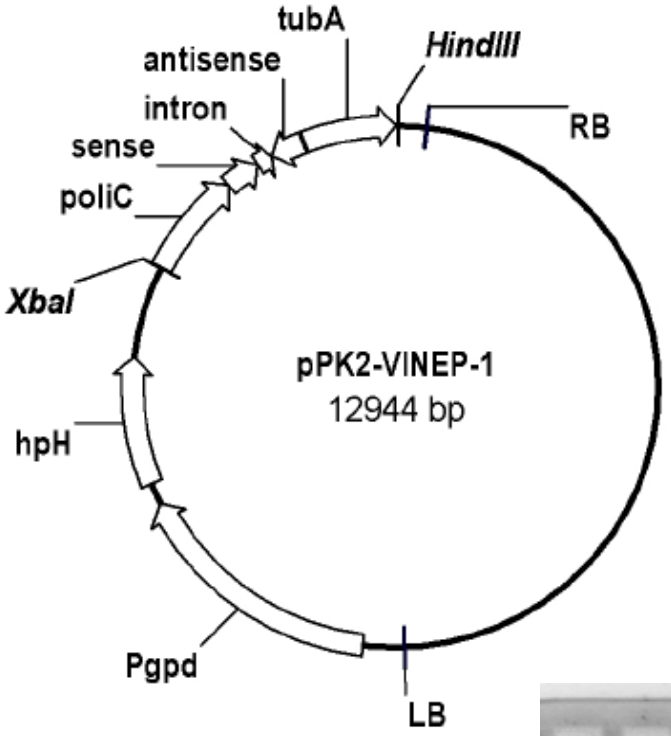
RNAi silencing of VlNEP-1

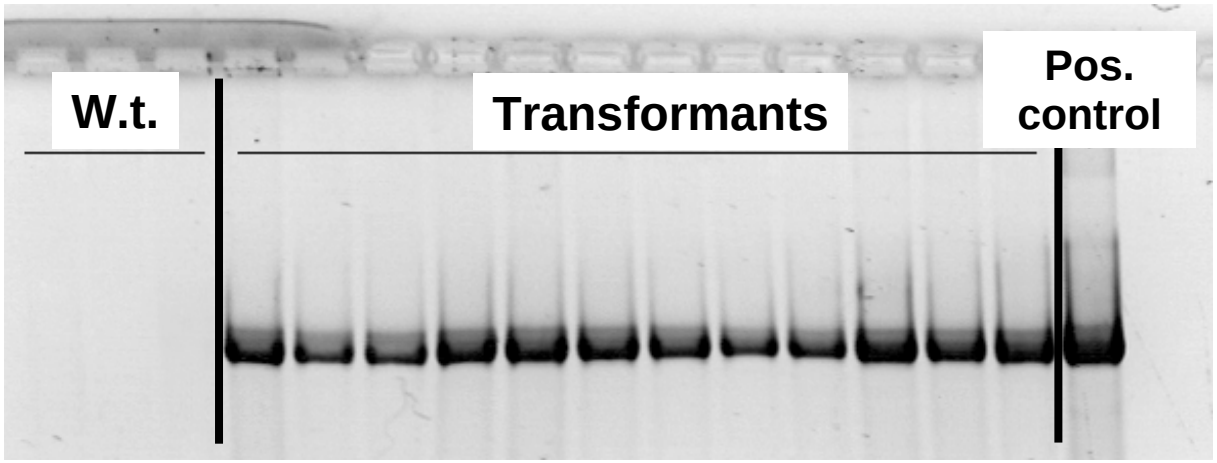


Malte

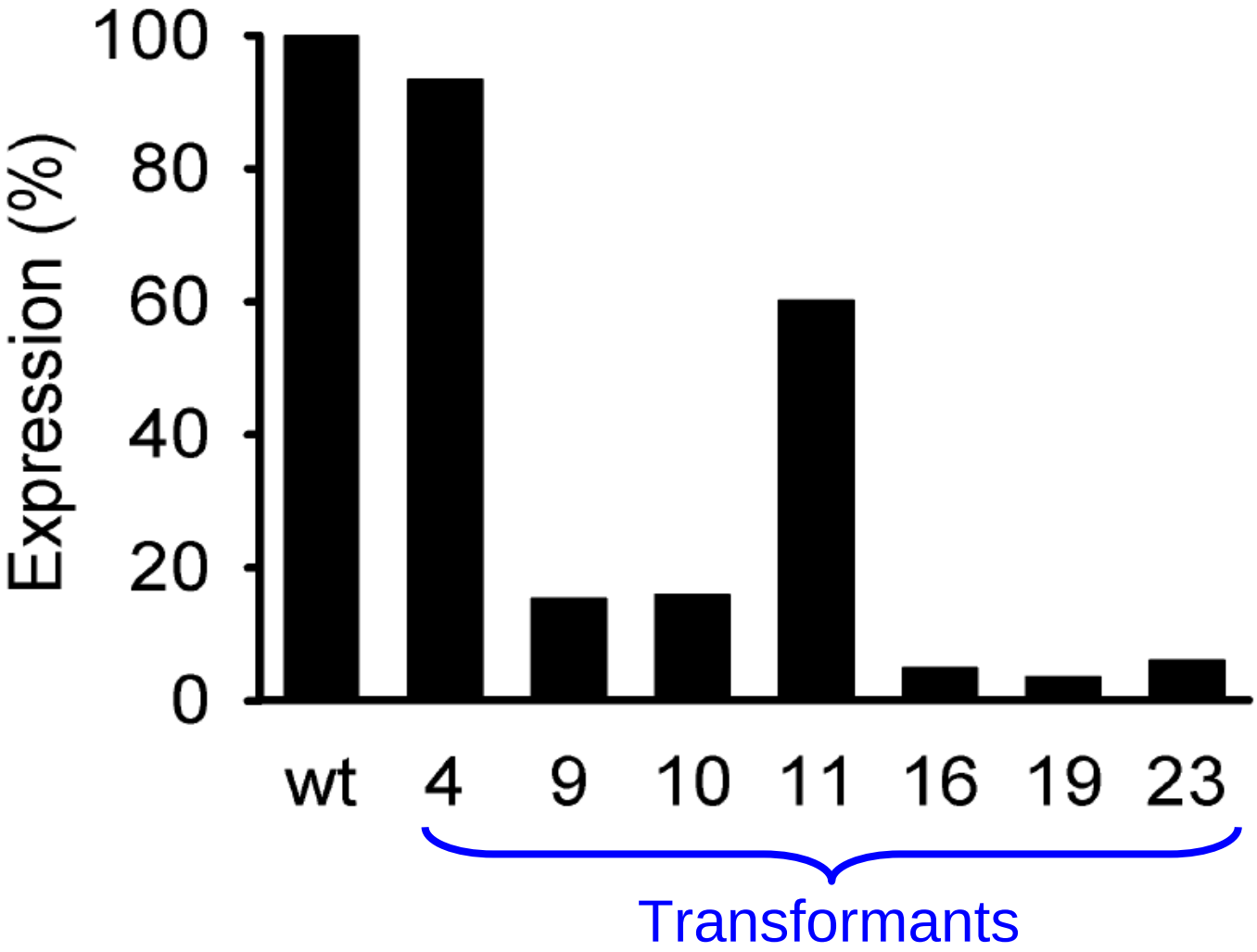
RNAi silencing of VlNEP-1: transformation



A. tumefaciens mediated genetic transformation



RNAi silencing of VlNEP-1: efficiency



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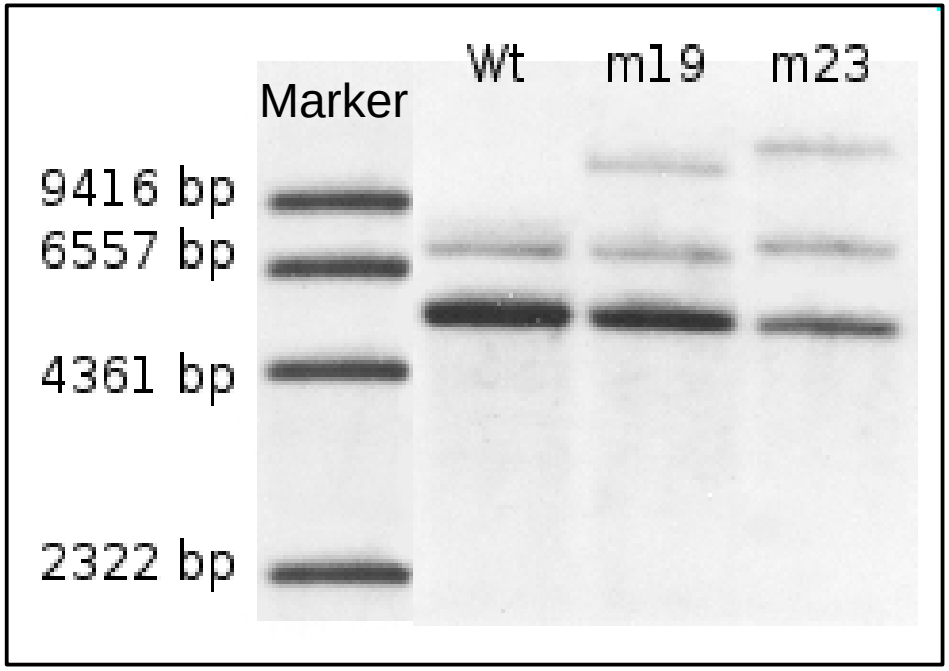
RNAi silencing of VlNEP-1: structure

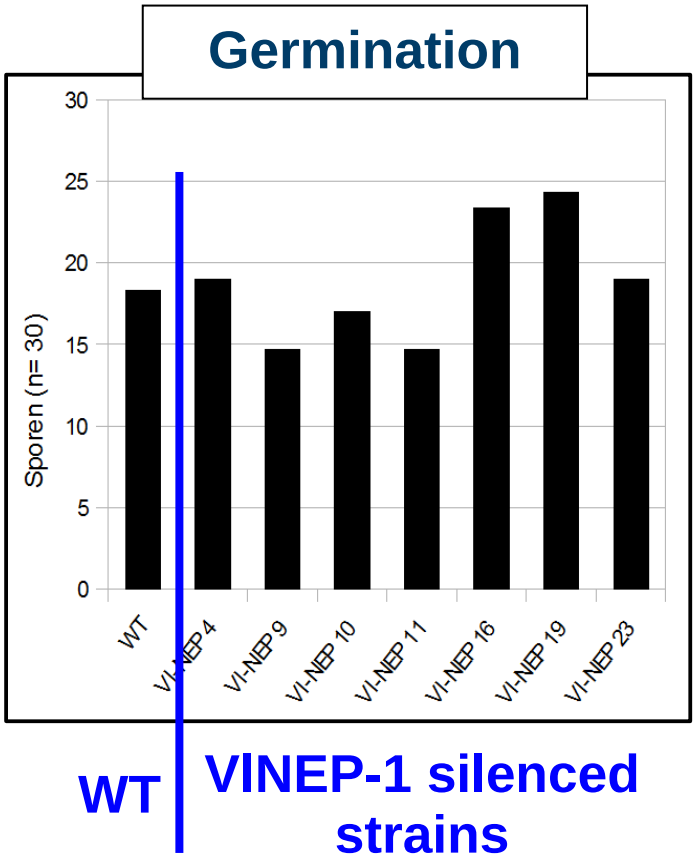
Single insertion of RNAi construct

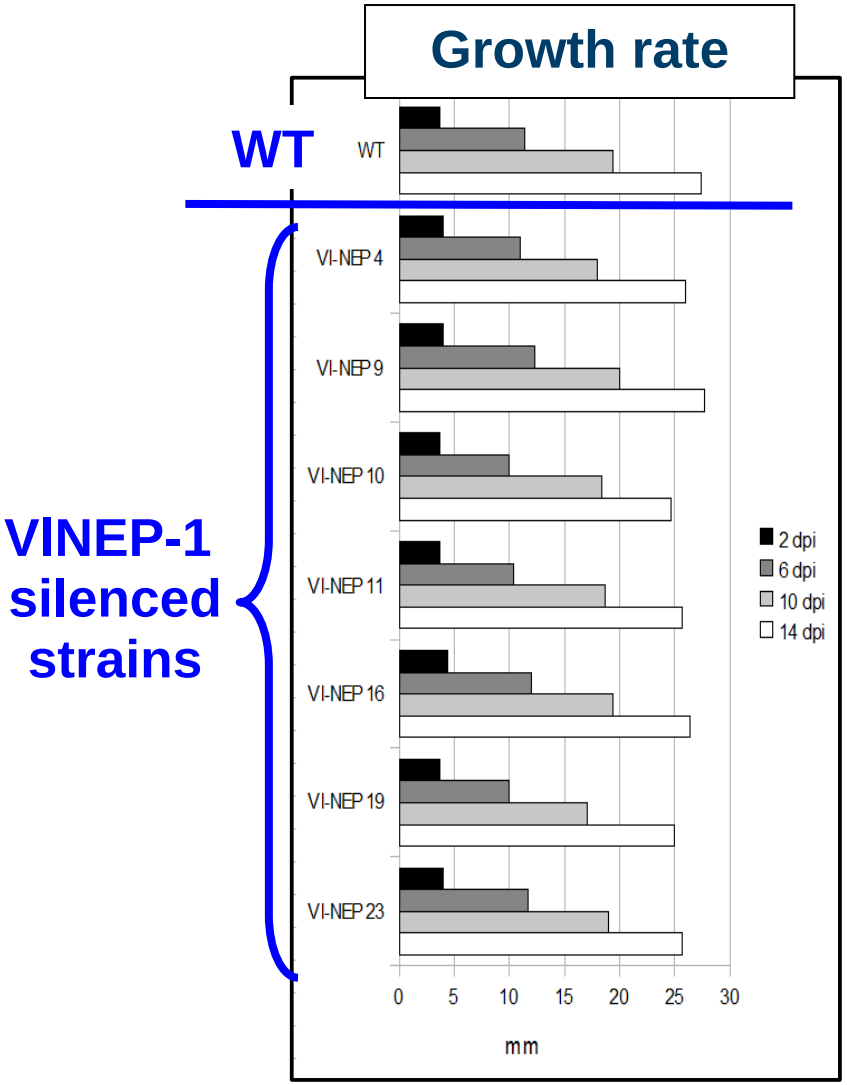


Southern hybridization with VlNEP-1 specific probe

Malte

Silencing VlNEP-1 does not affect phenotype





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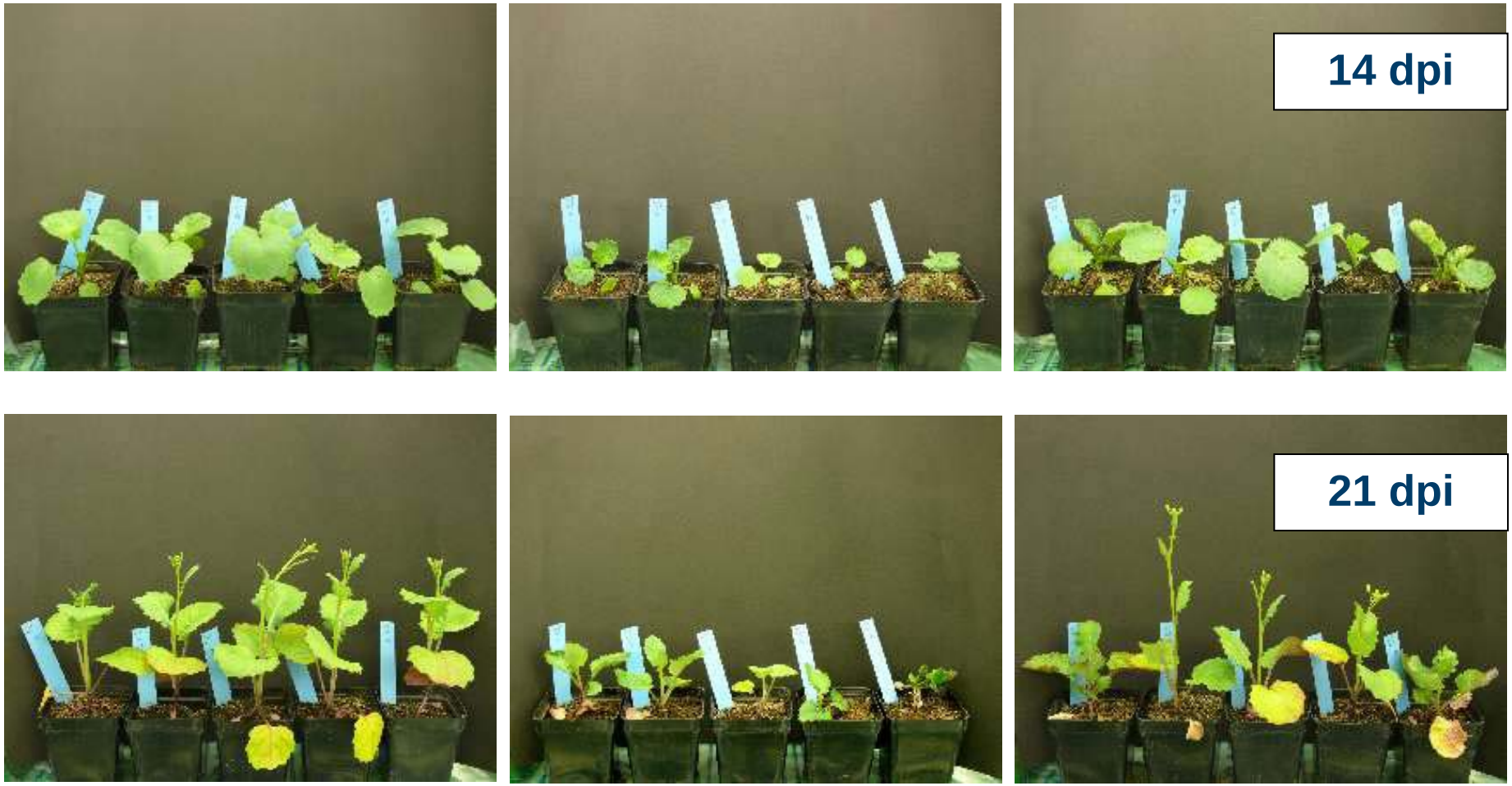
Silencing VlNEP-1 alleviates diseases symptoms

Control | **V.l. wildtype** | **V.l. VlNEP-1#23**



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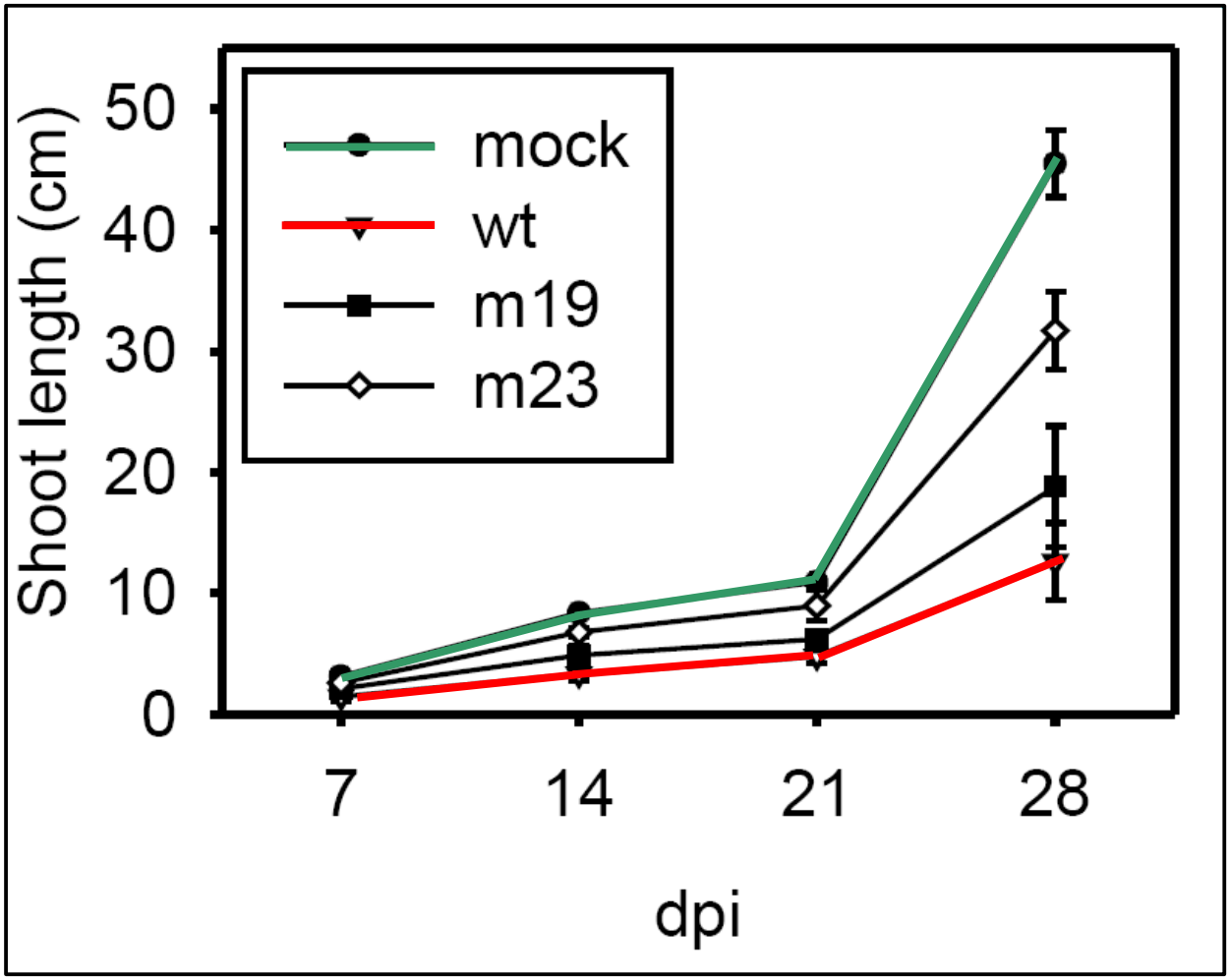
Silencing VlNEP-1 reduces stunting



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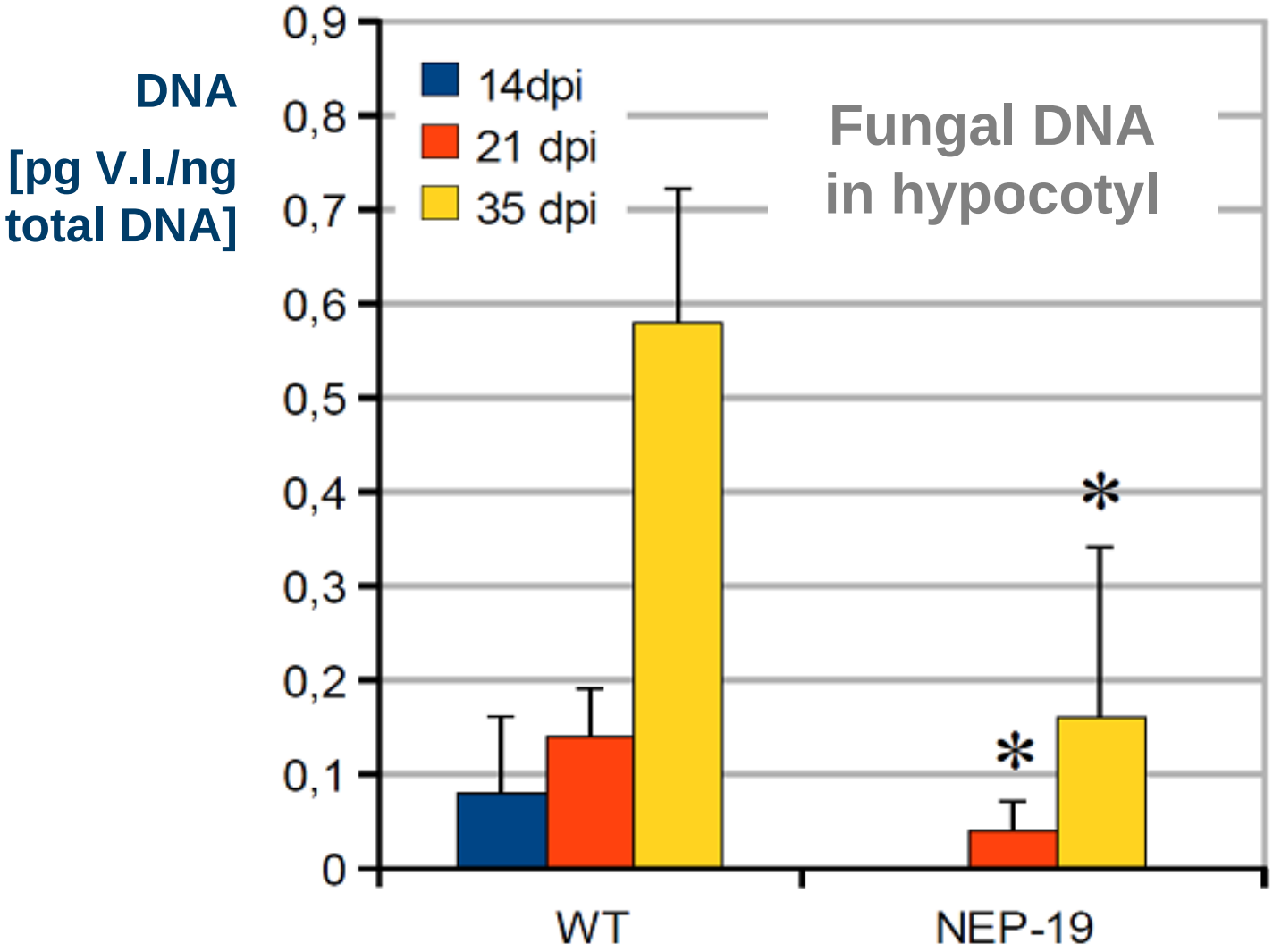
Silencing VlNEP-1 limits fungal growth in planta



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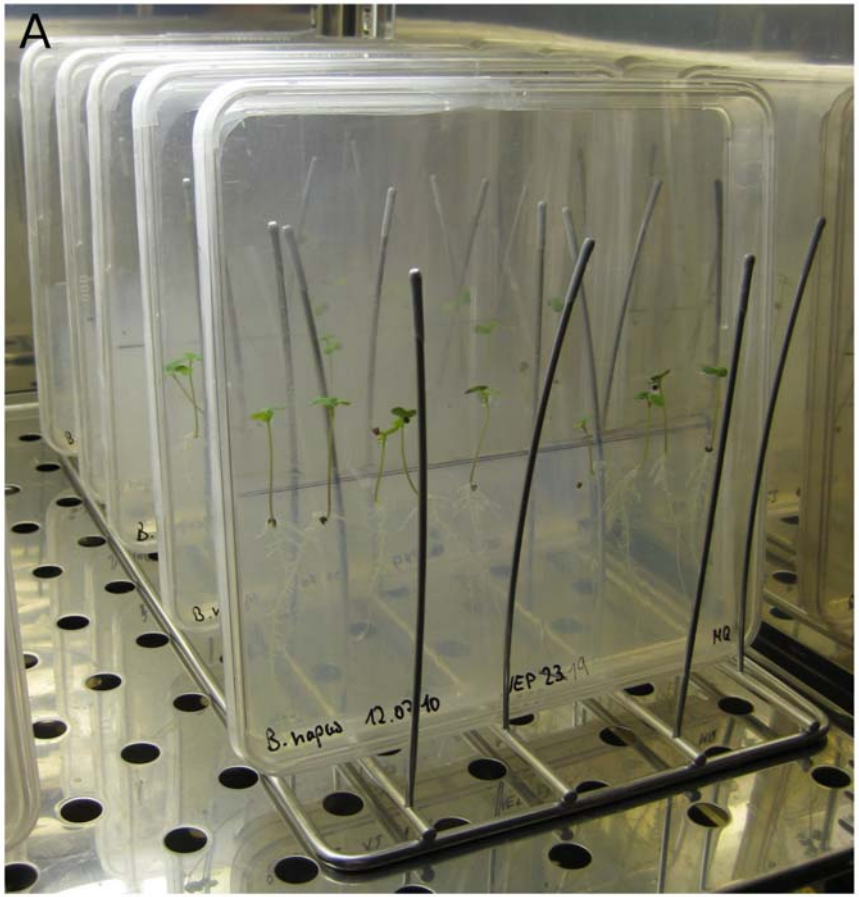
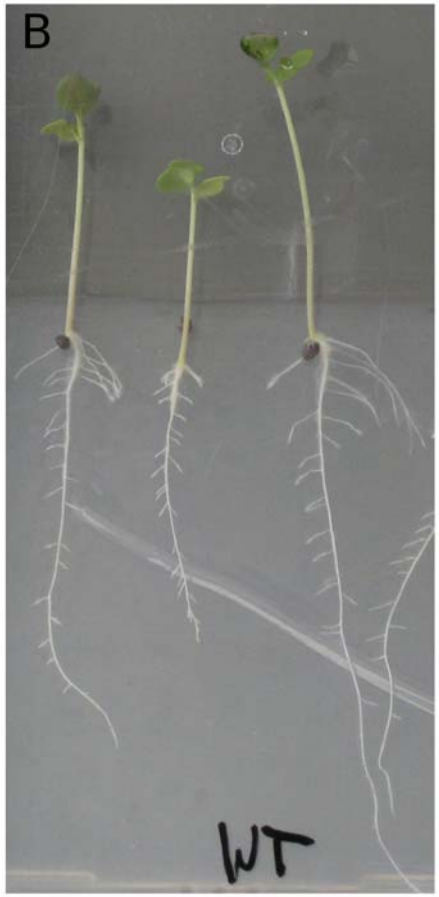
Histological study of root infection

B. napus grown on agar was inoculated with spores.



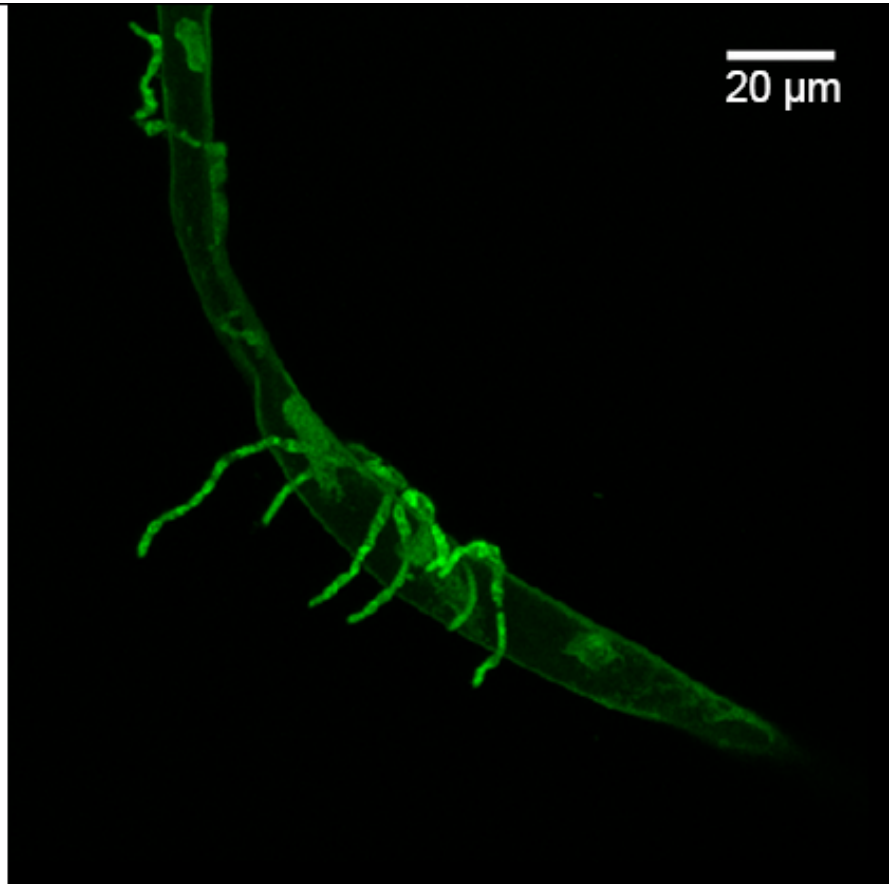


Mona

Infected roots of *Brassica napus* 12 hpi

V.l. wildtype

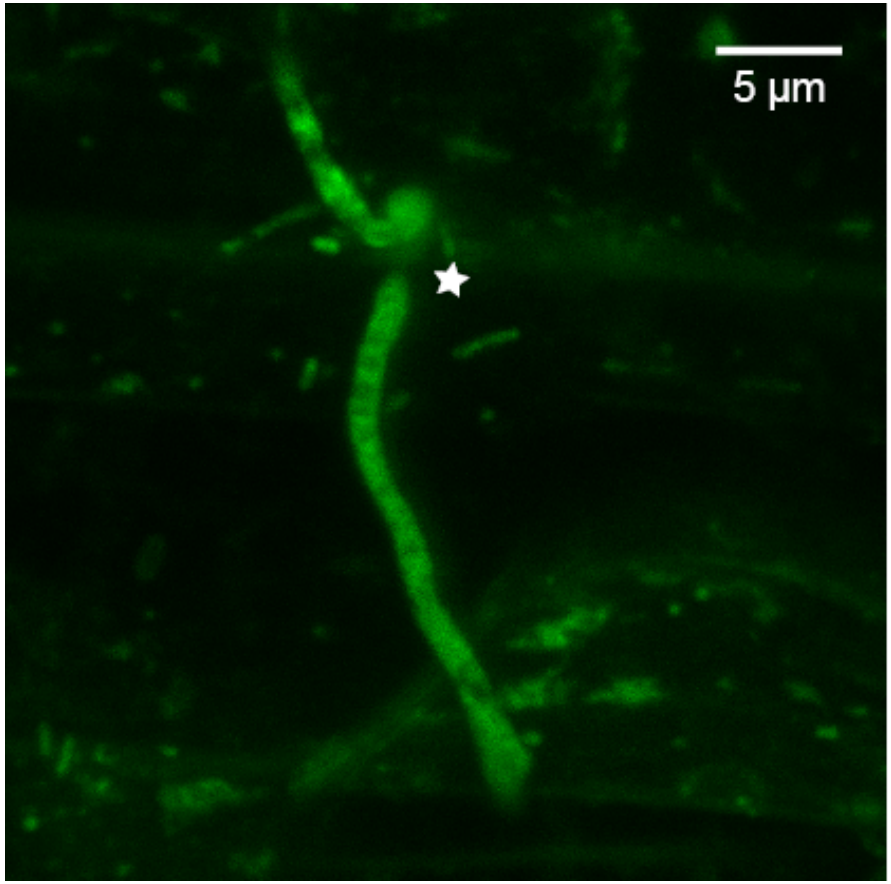
V.l. VlNEP-1#19



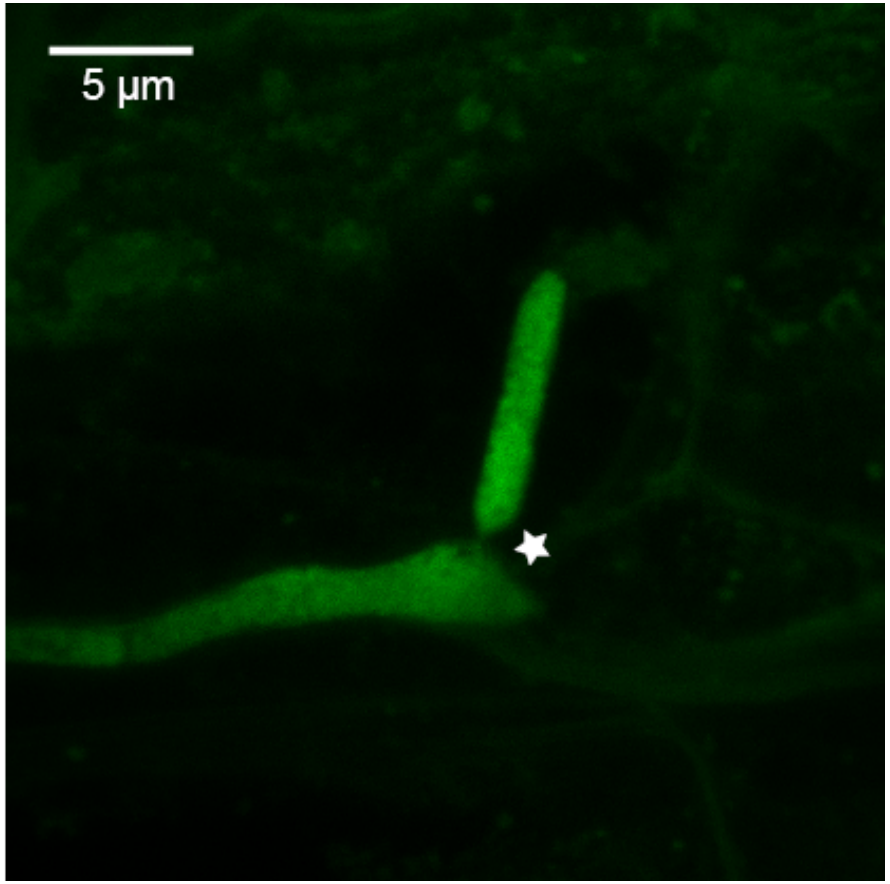
Mona

Infected roots of *Brassica napus* 72 hpi

V.l. wildtype

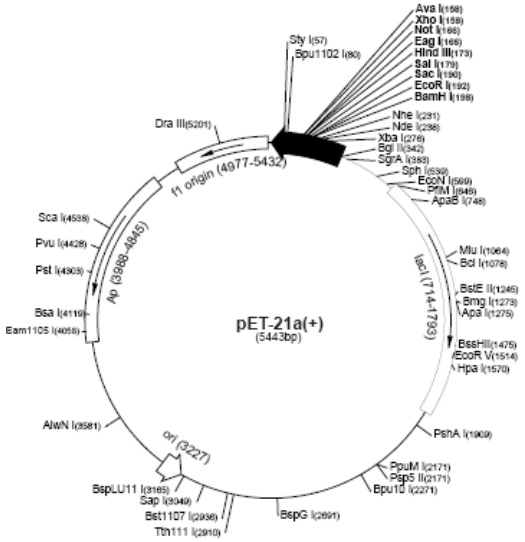


V.l. VlNEP-1#19

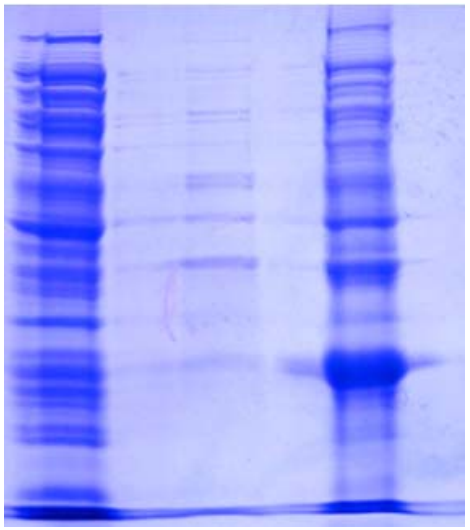


Mona

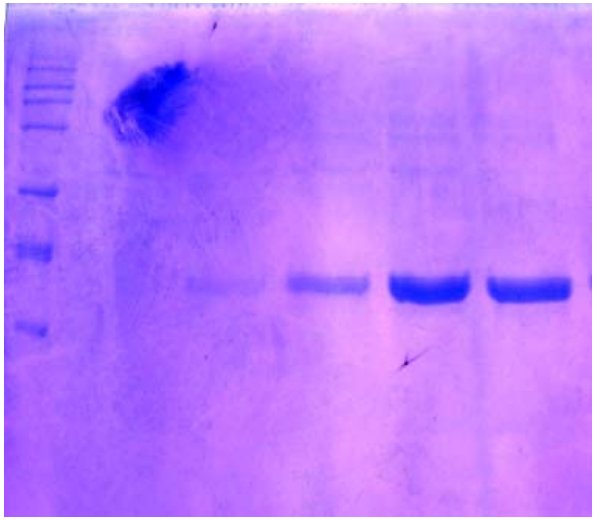
Purification of Vl-NEP-1 protein



Expression



Purification



Haidy & Ruth

Vl-NEP-1 causes leaf necrosis

Tobacco

B. napus

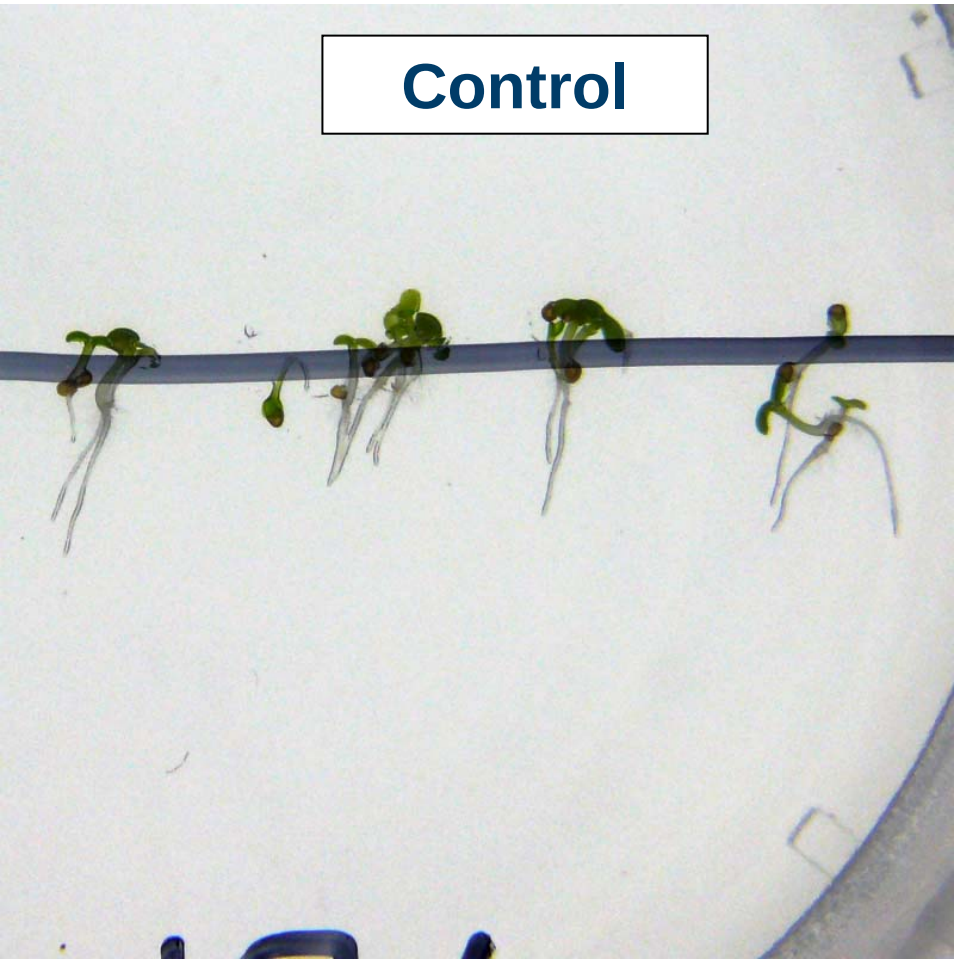
Native **Boiled** **Native** **Boiled**

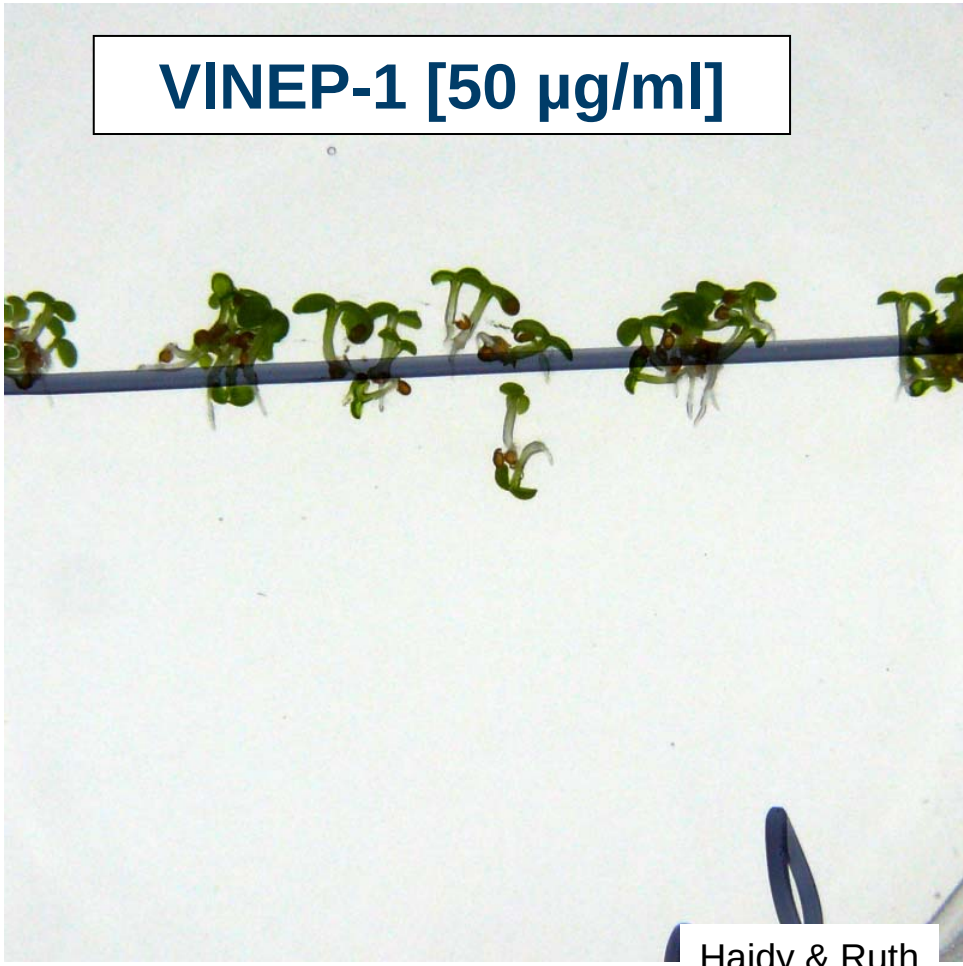
Infiltration of 100 µl of Vl-NEP-1 [50 µg/ml]

Haidy & Ruth

VlNEP-1 inhibits root growth in Arabidopsis

Arabidopsis thaliana LER (4 d)

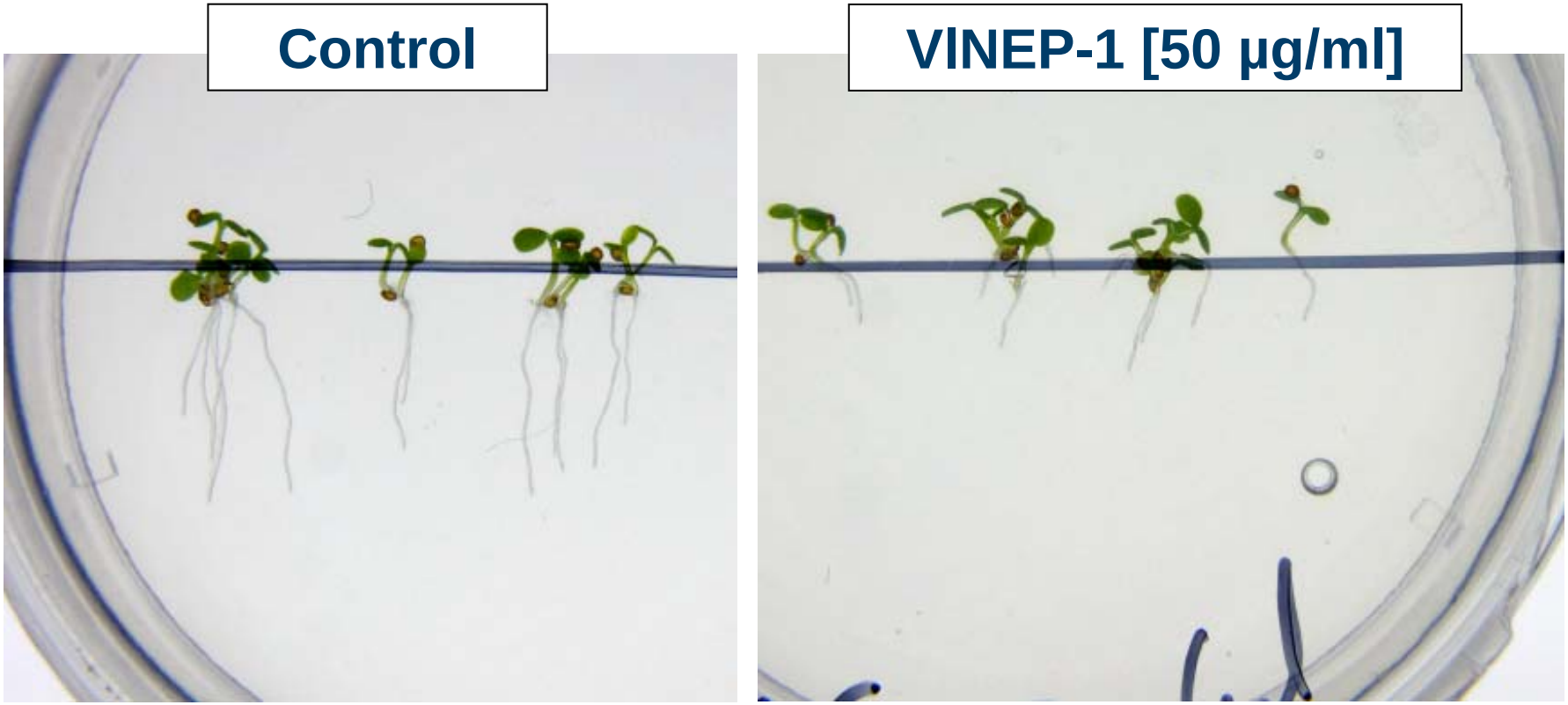




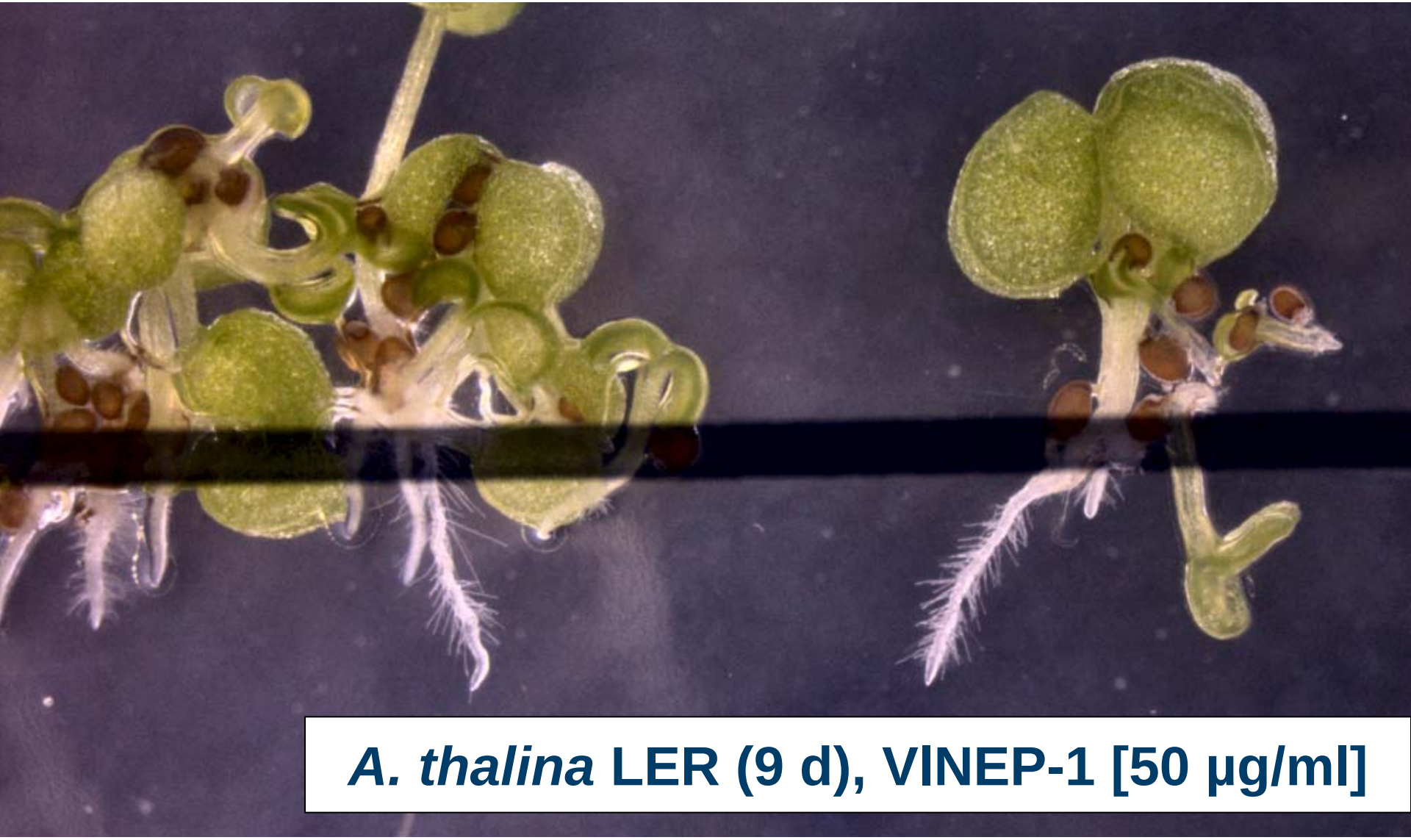
Haidy & Ruth

VlNEP-1 inhibits root growth in Arabidopsis

Arabidopsis thaliana BUR (4 d)



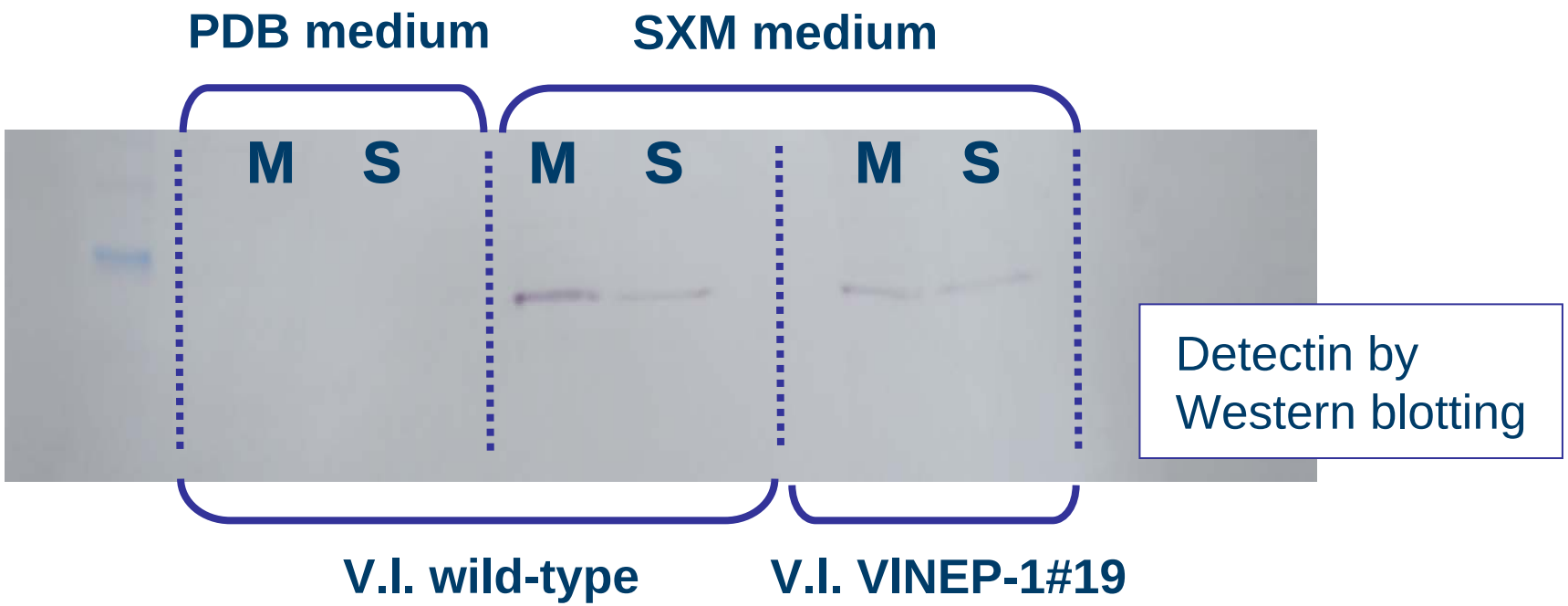
VlNEP-1 prevents germination of A.t. LER



A. thalina **LER (9 d), VlNEP-1 [50 µg/ml]**

Vl-NEP-1 is induced in xylem-simulating medium

M = mycelium
S = culture supernatant



Haidy & Ruth

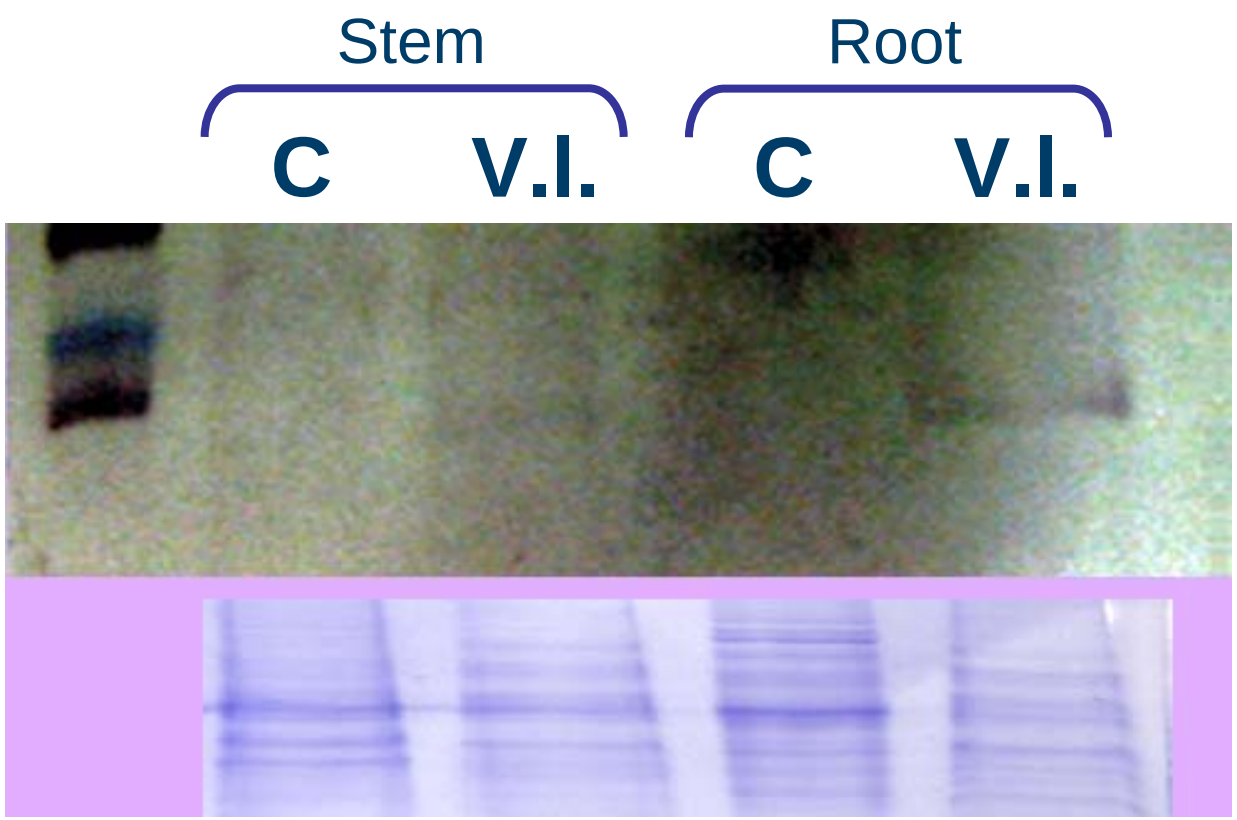
Fungus does not leave hypocotyl until 28 dpi

Tissue	Leaves	Hypocotyl
dpi	*ng VL-DNA/g FW*	*ng VL-DNA/g FW*
7	0.00 (±0.00)	2.88 (±0.89)
14	0.01 (±0.01)	9.94 (±2.79)
21	0.51 (±0.29)	3.95 (±1.65)
28	0.59 (±0.26)	10.41 (±1.52)
35	6.89 (±5.37)	25.58 (±6.79)

Eynck et al., 2007

VlNEP-1 is transported upwards in infected plants

B. napus 28 dpi with *V. longisporum*

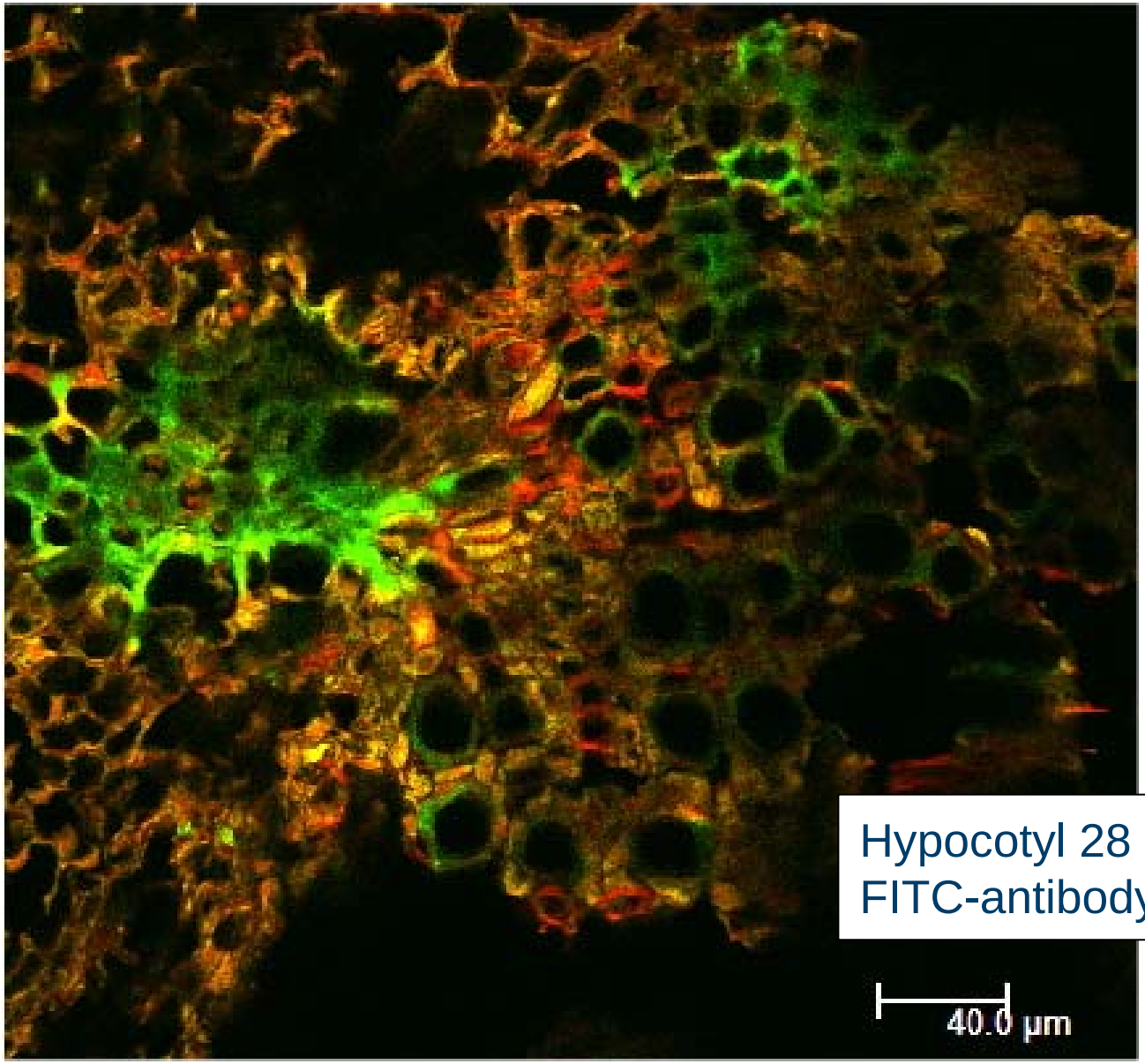


VlNEP-1 detected on Western blot

Total protein

Haidy & Ruth

VlNEP-1 in hypocotyl of infected *B. napus*



Hypocotyl 28 dpi,
FITC-antibody for VlNEP-1

Mona & Ruth

Conclusions

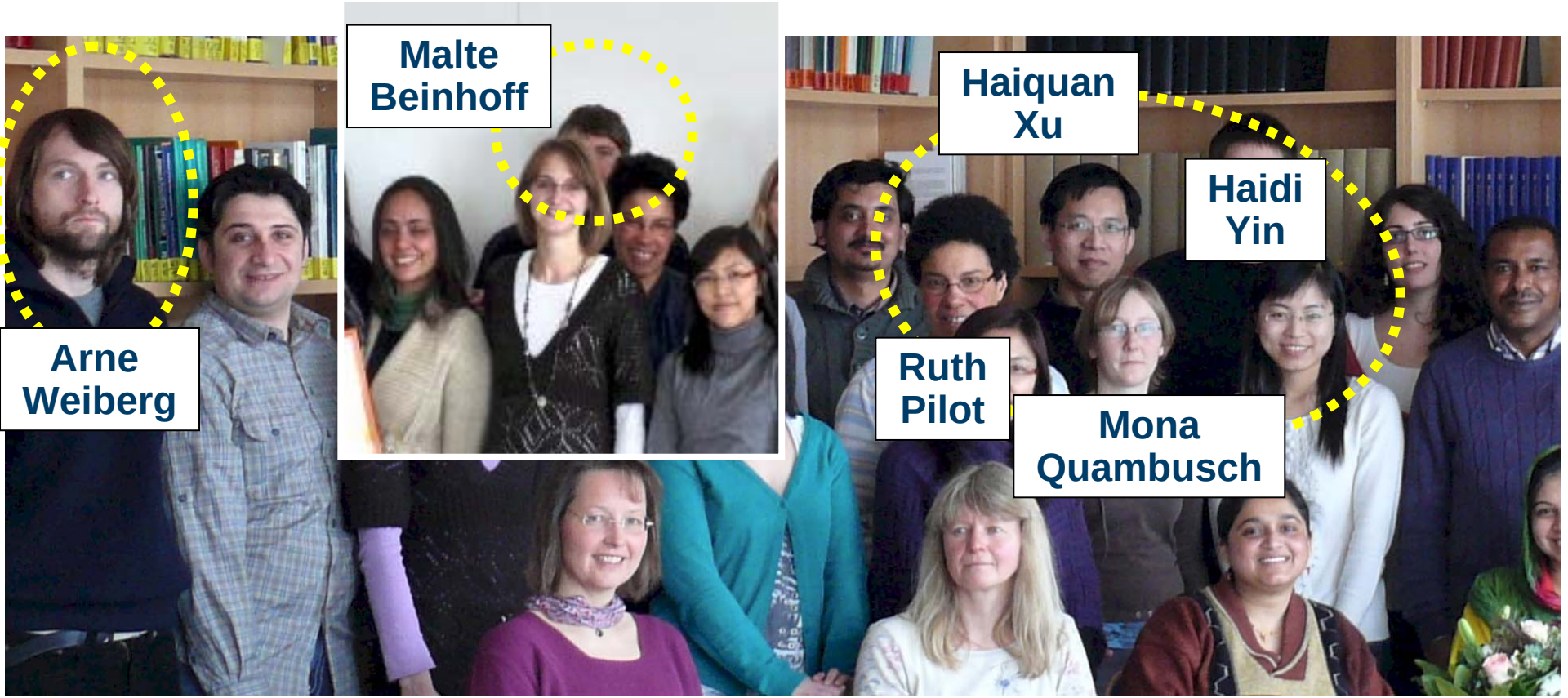
Findings

- Vl-NEP-1 is cytotoxic
- Vl-NEP-1 is strongly induced in planta
- Vl-NEP-1 increases fitness of V.l. in xylem
- Vl-NEP-1 does not effect root colonization

Hypothesis

- VlNEP-1 secreted in xylem causes leakage of nutrients from adjacent tissue

VINEP team


Arne Weiberg
Malte Beinhoff
Haiquan Xu
Haidi Yin
Ruth Pilot
Mona Quambusch