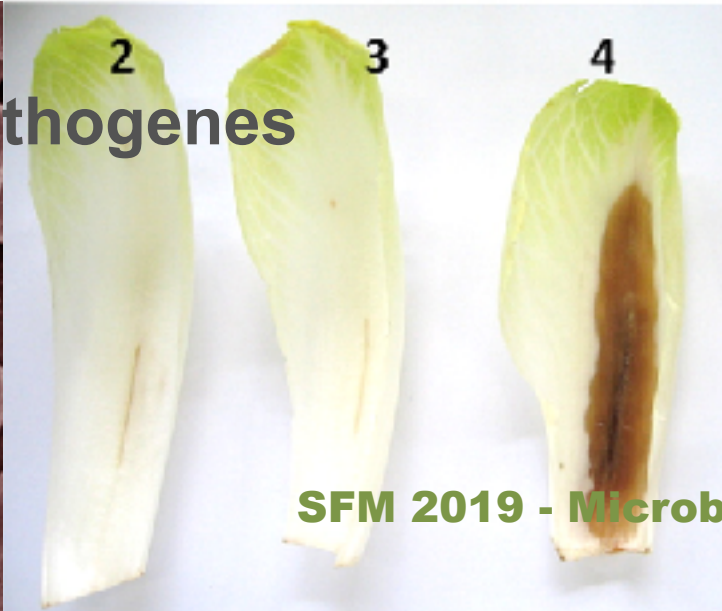
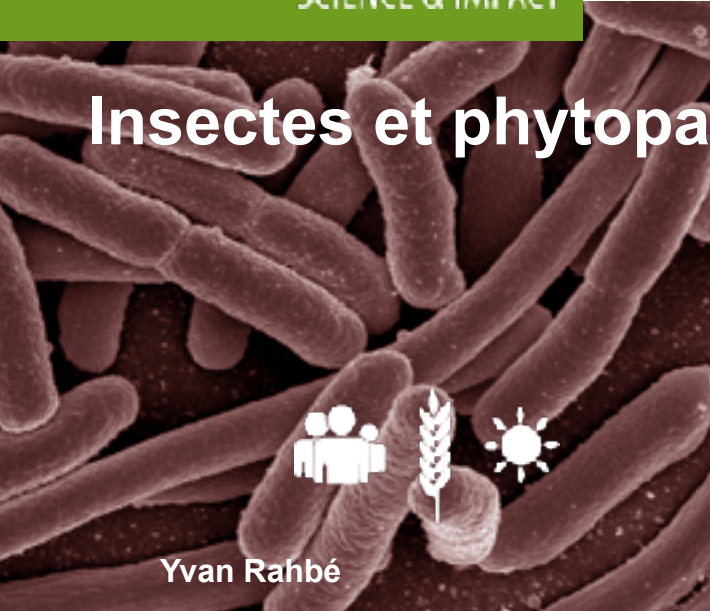
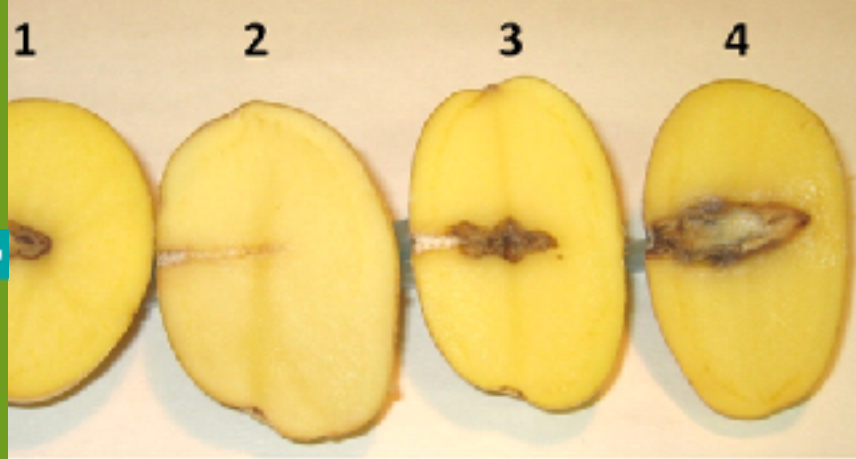


Le 1^{er} janvier, L'Inra et Irstea deviennent **INRAE**



INRA
SCIENCE & IMPACT



Insectes et phytopathogenes



SFM 2019 - Microbes'19 | 1/10/2019

Département Santé des Plantes



UMR5240 CNRS MAP (INSA de Lyon, UCBL, BayerCropScience)
Microbiologie Adaptation Pathogénie
Lyon

Bactéries pectinolytiques

INTRODUCTION - Topics

- Insect Physiology
- Hemipteran (plant-sucking) insects: Aphids
- Aphid symbiosis & microbial ecology:
 - aphid primary and secondary symbionts
 - « tertiary » players in aphid's « oligo » micro-ecosystem

INTRODUCTION - Topics

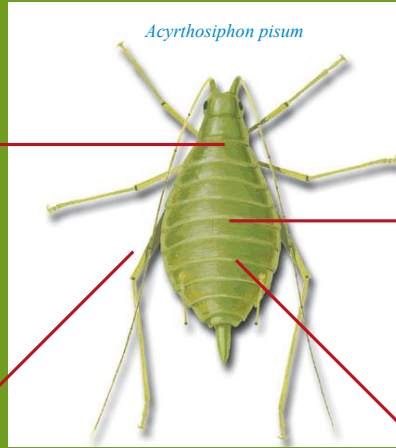
- Insect Physiology
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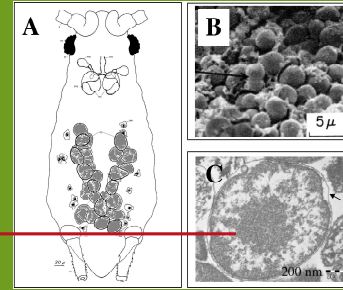
INTRODUCTION - Aphid interactions System



Aphidius ervi



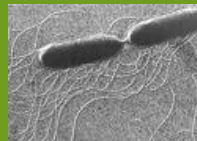
Acyrthosiphon pisum



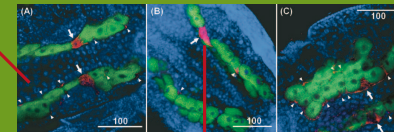
Dackosera aphidicola



Medicago truncatula



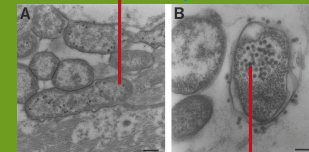
Sinorhizobium meliloti



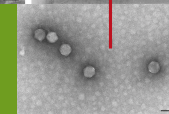
Serratia symbiotica

Regiella insecticola

Hemolymphic bacteria



Rickettsia sp.
Spiroplasma sp.



APSE2 phage

Talk map

- ❖ Part A : *Pathogénie, Symbiose, Vection*. Le grand tout du Phytobiome

- ❖ Terminologie de la symbiose d'insectes: S2, S2, S3

A

- ❖ Un pathogène de plantes adopte des entomotoxines

- ❖ Un pathogène de plante fait son coming out en co-symbiote primaire

- ❖ Part B: Mécanismes moléculaires, spécificités de la vection « non persistante »

- ❖ Terminologie et spécificités de la vection des phytovirus: NP, SP, C

B

- ❖ La cuticule: un solide biologique informé, Les CuPs (protéines cuticulaires)

- ❖ Chitine et autres signaux

C

- ❖ Part C: Insectes , Microbes et sensibilité au changement climatique

Typologie des symbioses d'insectes

	<i>Symbiose primaire</i>	<i>Symbiose secondaire</i>	<i>Symbiose tertiaire</i>	<i>Microbiote (autres)</i>
<i>Transmission</i>	Verticale	Verticale	Horizontale	Horizontale
<i>Prévalence (in species)</i>	Obligatoire	Facultative	Variable	Faible
<i>Charge microbienne</i>	++++	++	++	±
<i>Fonctions</i>	Trophique	Trophique Reproduction Défense	Variable	?
<i>Hôte alternatif</i>	Non	Non	Oui	Oui

A

Dickeya and insects - Starting from Bacterial genomics



Dickeya and insects - Starting from Bacterial genomics

JOURNAL OF BACTERIOLOGY, Apr. 2011, p. 2079–2077
 0021-9193/11/\$12.00 doi:10.1128/JB.11513-10
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Vol. 193, No. 5

Genome Sequence of the Plant-Pathogenic Bacterium *Dickeya dadantii* 3937^a

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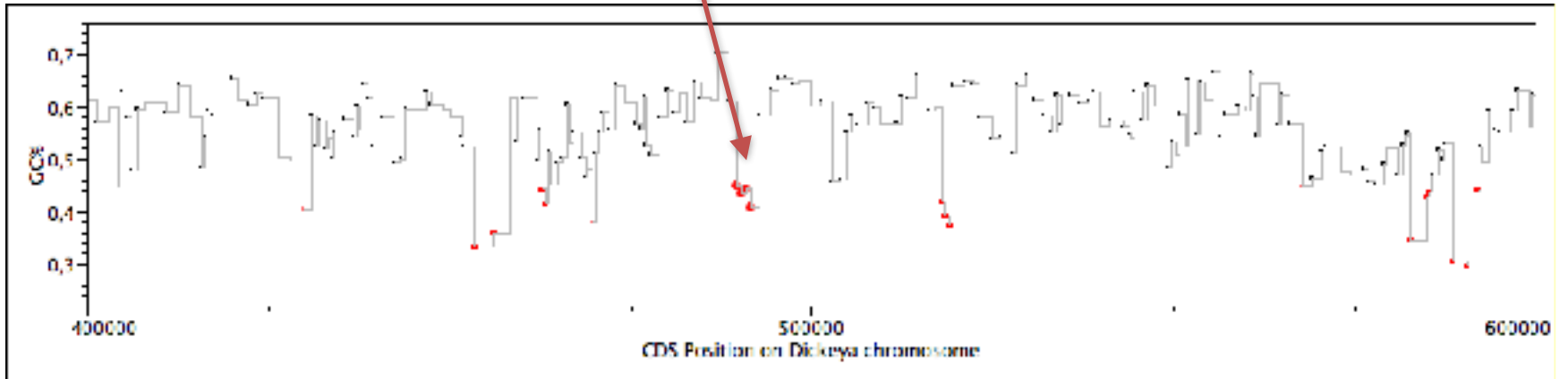
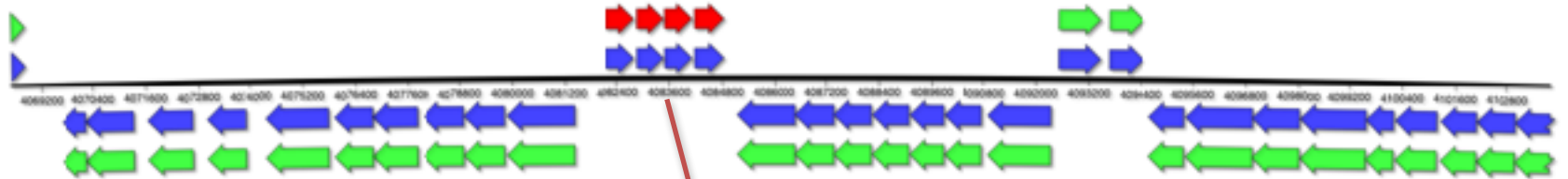
Received 16 December 2010/Accepted 23 December 2010

Dickeya dadantii is a plant-pathogenic enterobacterium responsible for the soft rot disease of many plants of economic importance. We present here the sequence of strain 3937, a strain widely used as a model system for research on the molecular biology and pathogenicity of this group of bacteria.

A

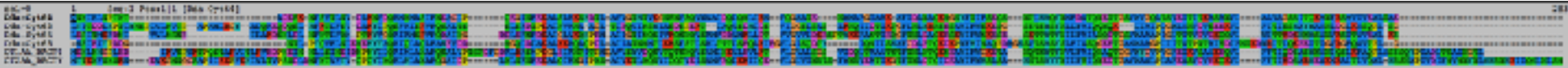


Cyt-like gene Cluster



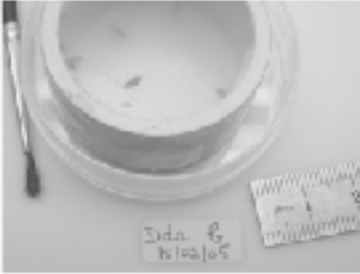
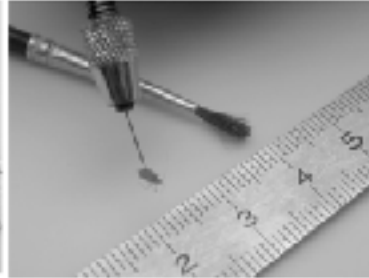
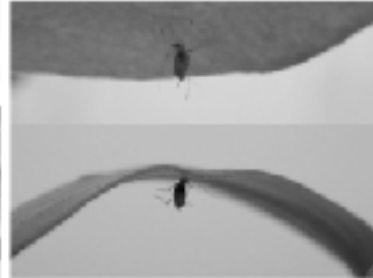
A

Dda-Cyt proteins (with Cyt1 Cyt2)



A

bacterial pathogen bioassays: ingestion, injection

A**B****C****D****E****F****G****H**

Grenier, A. M., G. Duport, S. Pages, G. Condemine, and Y. Rahbe. 2006. The Phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) Is a Pathogen of the Pea Aphid. *Appl. Environ. Microbiol.* 72: 1956-65.

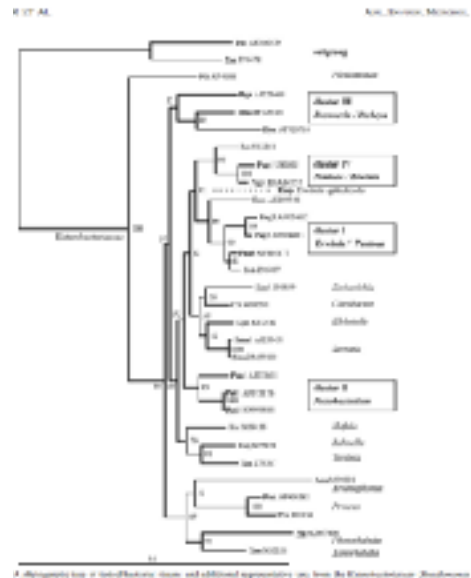
A Many enterobacteriaceae are pathogenic to aphids, among which *D.dadantii*

TABLE 1. Bacterial strains used in the present study, together with the relevant taxonomic information, previously published plant or insect interaction phenotypes, and their pathogenicity indexes (per oral and through acropic injury assays) on the pea aphid *A. pisum*.

Classification ^a	Bacterial species ^b	Abb.	Synonym	Strain ^c	EMBL accession no. (DSMZ record)	Pathogenicity ^d		ET ₅₀ ^e (day ± SE)		Reference(s) of phenotype ^f		
						Phyto +/-	Tarsonix +/-	Ingestion ^g	Injection			
Enterobacteriaceae	<i>Erwinia amylovora</i>	Ura1	<i>Erwinia amylovora</i>	CHP 805 = 807	AJ558937	+	– (partial)	–	2.56 ± 0.19	0.66 ± 0.13	30	
		Ura2	<i>Erwinia amylovora</i>	Ura2 (repeated)	AJ558937	–	–	–	2.36 ± 0.26	0.62 ± 0.13	32	
		Dpa	<i>Erwinia amylovora</i> sp. parvifoliae	CHP 817	AJ928786	+	+++	–	2.30 ± 0.35	1.26 ± 0.13	17	
	<i>Erwinia garmanii</i>	Spa	<i>Erwinia garmanii</i>	CHP 847	AJ223446	+	–	–	8.81 ± 0.76	1.48 ± 0.13	6	
		Tap	<i>Erwinia garmanii</i>	CHP 829	CHP 829	+	1 (partial)	–	9.00 ± 0.55	0.54 ± 0.13	3	
	<i>Erwinia caroliniana</i>	Tad	<i>Erwinia caroliniana</i>	CHP 2686	AJ223441	+	– (partial)	–	1.56 ± 0.17	0.75 ± 0.14	3	
		Pa2	<i>Erwinia caroliniana</i>	CHP 212	AJ223441	+	8 (partial)	+++	1.88 ± 0.14	1.88 ± 0.23	8	
	<i>Erwinia straelii</i>	Pa1	<i>Erwinia straelii</i>	UMG collection	AF931136	+	–	–	4.15 ± 1.09	1.44 ± 0.13	5	
		Pa2	<i>Erwinia straelii</i>	ISIRI 1647	U999821	+	–	–	5.23 ± 0.33	0.50 ± 0.05	5	
	<i>Erwinia carovora</i>	Era	<i>Erwinia carovora</i>	SCRI 45421	EF907	+	–	–	–	1.46 ± 0.13	37	
		Pa	<i>Erwinia carovora</i>	CHP 814	Y1121	+	–	–	2.17 ± 0.64	0.58 ± 0.13	1	
	<i>Erwinia amylovora</i>	Pa2	<i>Erwinia amylovora</i>	CHP 818	U3075	+	–	–	9.10 ± 0.38	1.75 ± 0.13		
		Pa2	<i>Erwinia sp.</i> , strain TAC1	TAC XII 99.2	AJ924603	–	Symbiont (strip)	–	2.12 ± 0.17	0.30 ± 0.05	14, 15	
		Pa2	<i>Erwinia sp.</i> , strain TAC2	TAC XII 99.3	AJ924602	–	Symbiont (strip)	–	1.36 ± 0.13	0.50 ± 0.05	14, 15	
Pa2		<i>Erwinia sp.</i> , strain TAC10	TAC 98.2	AJ516173	–	Symbiont (strip)	–	2.50 ± 0.13	0.50 ± 0.05	14, 15		
Eubacteriales	<i>Enterobacter coli</i>	Ecol1	<i>Enterobacter coli</i>	NL1-Bio (Strasbourg)	20889	–	–	–	–	–	12	
		Ecol2	<i>Enterobacter coli</i>	NMS2 (Paris) (type strain)	AJ576275	–	–	–	4.03 ± 0.71	1.50 ± 0.13	36	
		Ecol3	<i>Enterobacter coli</i>	Tap 11 (bio) (type strain)	–	–	–	–	4.17 ± 0.33	1.81 ± 0.13	36	
		Ecol4	<i>Enterobacter coli</i>	E121	–	–	–	–	5.00 ± 0.42	3.78 ± 0.07	–	
		Pa1	<i>Enterobacter coli</i>	UMG collection	AJ930493	–	–	–	8.10 ± 0.59	0.40 ± 0.13	19	
		Sara1	<i>Enterobacter coli</i>	UMG collection	AJ223431	+	–	–	2.62 ± 0.21	0.50 ± 0.05	39, 40	
		Sara2	<i>Enterobacter coli</i>	ISIRI 267	AJ99361	+	–	–	1.05 ± 0.13	0.50 ± 0.05	39	
		Sara3	<i>Enterobacter coli</i>	ISIRI 1	AJ99361	+	(partial)	–	1.98 ± 0.14	0.50 ± 0.05	39, 52	
		Sara4	<i>Enterobacter coli</i>	ISIRI 1	AJ99361	+	–	–	9.01 ± 0.38	0.51 ± 0.05	39, 52	
		Sara5	<i>Enterobacter coli</i>	Y101	AJ307484	–	+	(partial)	–	–	1.42 ± 0.13	29
		Sara6	<i>Enterobacter coli</i>	X1	AJ223431	–	–	–	–	1.32 ± 0.14	25, 25	
		Sara7	<i>Enterobacter coli</i>	UMG 1281	AJ930491	–	–	–	–	–	–	26
		Sara8	<i>Enterobacter coli</i>	ATCC 4511	–	–	–	–	–	–	–	–
<i>Paenibacillus</i>	<i>Paenibacillus arvensis</i> sp. <i>arvensis</i>	Pa	<i>Paenibacillus arvensis</i> sp. <i>arvensis</i>	CHP 212 (type strain)	AJ223439	+	(partial)	–	6.21 ± 0.44	1.41 ± 0.13	7	
		Pa	<i>Paenibacillus arvensis</i> sp. <i>arvensis</i>	CHP 2686 (type strain)	X7672	[+]	+	(weak pathogen)	–	2.10 ± 0.19	0.68 ± 0.13	8, 22, 28

^a Taxonomic information was taken from strain providers, mainly CHP, and checked for the recent changes in the “Erwinia” group taxonomy (2018). “Erwinia” clusters were re-assigned by Hansen et al. (35) and Lindy et al. (21), which were corroborated with our phylogenetic analyses. Clust. abbrev.:

^b Sequenced genomes are in boldface.
^c All abbreviations used in our text and figures.
^d Strain information is given based on: (i) taxonomic/phenological position (CHP, UMG, and ISIRI), see Materials and Methods; with additional strain nomenclature for fully sequenced genotypes and literature reference or commercially available strains (ATCC/Ecol);
^e Pathogen phenotype previously known and published (see footnote g) for the strains used in this study. +, known pathogen (for the green leafy); –, tested negative pathogen/blank or N/D, unreported phenotypes for the given host or system. The taxonomic column (strain provider) does not refer to insect infection in the screening of Bross et al. (25).
^f Multiple phenotypes are quantified from the presence of plant necrosis (ET₅₀, 50% lethal time [median survival time]). Data to the high doses used (i.e. dose = 10⁷ bacteria/ml) and to the dose response curves obtained on the limited set of host analyzed in more detail, bacteria with a pathogenicity index higher than 3 days are uniformly considered comparable to weak pathogens.
^g Select direct or indirect references for the main pathological phenotypes of the strains used in our study (see the appropriate section of Results for our rationale of strain selection and phylogenetic analyses).



A Phylogenetic tree of Enterobacteriaceae strains and additional representatives from the Eubacteriales (Bacteroidetes).

A

The pathogenicity of *Dda* is somehow insect-specific

TABLE 2. Pathogenicity assays for *D. dadantii* (*E. chrysanthemi* 3937) in insects from four different orders

Insect species	Insect order	<i>D. dadantii</i> administration			
		Needle injection		Ingestion	
		% Mortality (n)	Mean LT ₅₀ (days) ± SE	% Mortality (n)	Mean LT ₅₀ (days) ± SE
<i>A. pisum</i>	Hemiptera	100 (30)	0.6 ± 0.11	100 (30)	2.36 ± 0.24
<i>D. melanogaster</i>	Diptera	0 (90)		0 (120)	
<i>S. littoralis</i>	Lepidoptera	0 (40)		0 (40)	
<i>S. oryzae</i>	Coleoptera	60 (24)	3.0 ± 0.86	0 (24)	

Grenier, A. M., G. Duport, S. Pages, G. Condemine, and Y. Rahbe. 2006. The Phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) Is a Pathogen of the Pea Aphid. *Appl. Environ. Microbiol.* 72: 1956-65.

A the Δ cyt (cyt cluster deletion) mutant is highly altered in oral pathogenicity

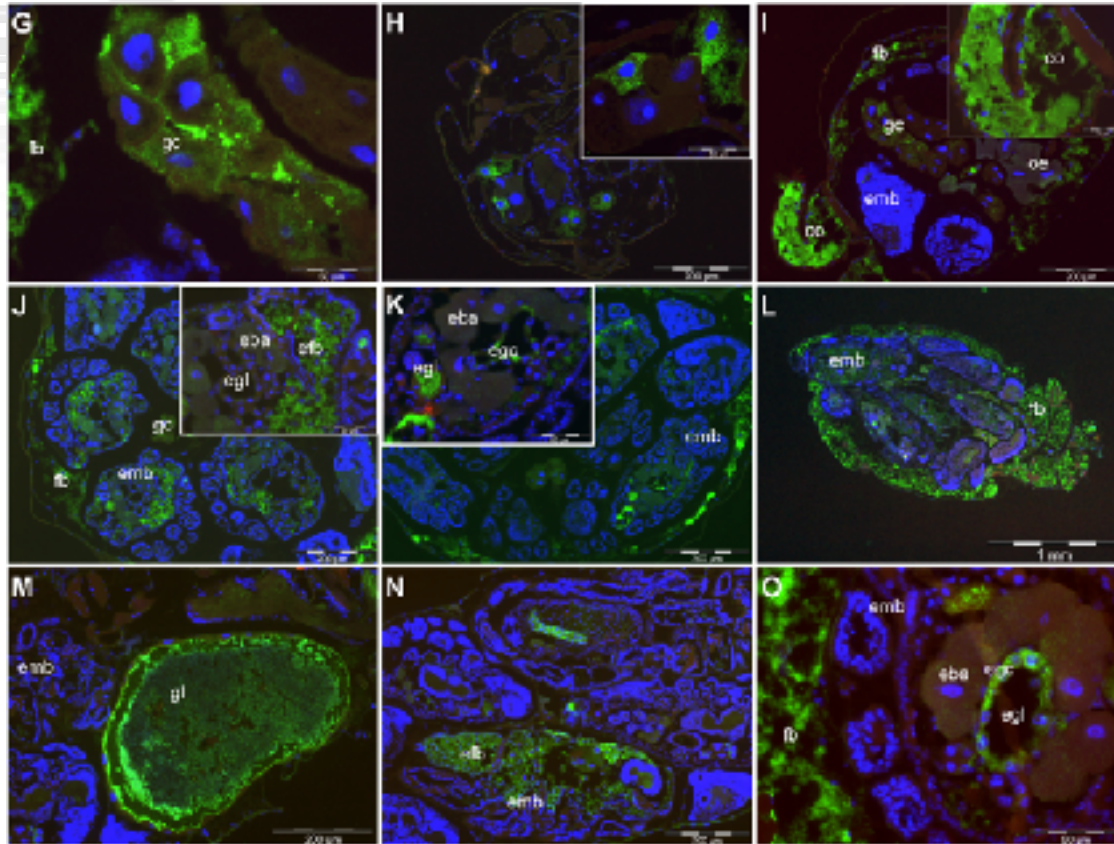
TABLE 3. *A. pisum* virulence assays for *D. dadantii* (*E. chrysanthemi* 3937) wild-type strain and A4664 mutant (Δ cytABCD) after septic inoculation or oral challenge at different doses

Assay and dose (bacteria/ml)	LT ₅₀ ± SE (days)		Log-rank test result (P) ^a
	<i>D. dadantii</i> wild type	<i>D. dadantii</i> Δ cyt mutant	
Injection	1.4 ± 0.13	1.4 ± 0.14	0.75
Ingestion			
10 ⁷	2.1 ± 0.25	3.0 ± 0.97	0.001
10 ⁶	2.5 ± 0.16	5.2 ± 0.75	<0.0001
10 ⁵	2.5 ± 0.13	3.9 ± 0.40	<0.0001
10 ⁴	2.8 ± 0.88	>8	0.019

^a Wild-type versus mutant survival comparison.

septicemia and infected tissues

A



A



expression of toxin CytC

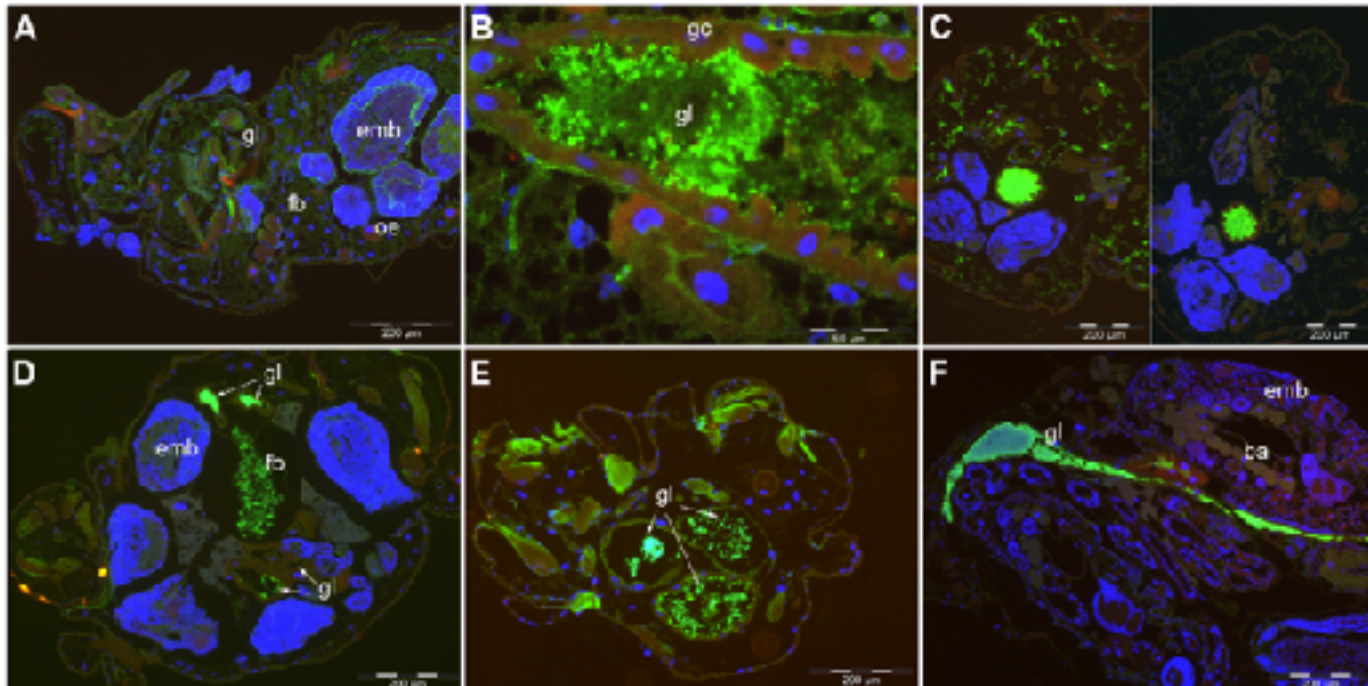


Figure 3. Expression of CytC toxin in aphid tissues. Immunostaining with anti CytC antibodies (except 3C, left) of aphids infected with the wild type *D. didactyli* strain. Anti CytC antibodies were detected with anti IgG labeled with Alexa fluor 488 (green labeling) and DNA was stained with DAPI (blue labeling). **3A to 3B:** early infection stage; day 1 of infection. In most samples, no expression of the toxin was detected (3A) although bacterial presence was readily observed in the same sample, for example in the gut lumen (see Fig. 2); in some individual insects, toxin expression was detected in the gut lumen (3B). **3C to 3E:** Primary infection stage; day 2 post infection; 3C, left and right: typical staining difference (in the same aphid) between bacterial detection at many locations, including the fat body (anti-KdgM antibody, 3C left) and toxin expression detection, restricted to the digestive tract (anti-CytC antibody, 3C right); in some instances, toxin expression was also detected in clustered areas of the fat body, in addition to typical gut lumen localization (gl, fb, 3D-E); **3F:** late infection stage, day 4 post-infection. Toxin detected as a marked stain only in the gut lumen (3F). Abbreviations as in Figure 2, plus: oec: pericytes.
doi:10.1371/journal.pone.0030702.g003

A operon regulation by temperature, osmolarity (sucrose) and regulator mutants

Table 1. Expression of the *cytA::lacZ* fusion *in vitro* for a set of regulator gene mutations.

Growth condition ^a	23°C		34°C	
	M63Y	+ sucrose 10%	M63Y	+ sucrose 10%
Genetic background				
wt (A4684)	46 ± 8 [B,cd] [γ] ^b	98 ± 27 [C,c] [αβ]	83 ± 11 [C,ab] [β]	106 ± 38 [C,ab] [α]
<i>hns</i> (A4926)	650 ± 131 [A]	4404 ± 501 [A]	2253 ± 459 [A]	12106 ± 059 [A]
<i>vimE</i> (A4930)	585 ± 103 [A]	3332 ± 538 [B]	303 ± 166 [E]	4206 ± 988 [B]
<i>pecD</i> (A5080)	33 ± 4 [B,e]	70 ± 11 [C,a]	63 ± 6 [C,c]	86 ± 36 [C,b]
<i>piroP</i> (A5032)	54 ± 7 [B,ab]	85 ± 25 [C,a]	77 ± 12 [C,b]	96 ± 16 [C,ab]
<i>gacA</i> (A5084)	41 ± 5 [B,d]	99 ± 40 [C,a]	84 ± 11 [C,ab]	130 ± 31 [C,a]
<i>ompR</i> (A5129)	51 ± 3 [B,bc]	99 ± 12 [C,a]	93 ± 11 [C,a]	146 ± 11 [C,e]
<i>kstR</i> (A5187)	46 ± 3 [B,bcd]	nd	nd	nd
<i>pecT</i> (A5188)	64 ± 4 [B,a]	nd	nd	nd

a. The expression of the *cytABCD* operon was measured in M63Y medium supplemented, or not, with 10% sucrose at 28°C or 34°C as the growth conditions. The β-galactosidase activities result from at least five independent repeats per modality ($n = 5-18$) and are expressed in μmol of o-nitrophenol produced per minute and per milligram of bacteria ± standard deviation.

b. ANOVA with complete three-level factorial design identified the following order of underlying factors for *cytABCD* operon expression: (i) genetic ($P < 10^{-166}$), (ii) medium ($P < 10^{-99}$) and (iii) temperature ($P < 10^{-13}$). All interactions being significant, we analysed separately the genetic factor within the split medium × temperature design (above). Variance inhomogeneity between high-strength regulators (*hns* and *vimE*) and other genes precluded precise mean analysis of the low-strength regulators (note: non-parametric ANOVA – Kruskal-Wallis test – resulted in identical conclusions). We therefore split the ANOVA analyses between a global analysis identifying the effects of *hns* and *vimE* (capital letter groupings; by columns: groups sharing the same letter did not differ under a Tukey HSD test at 5% confidence level) and a subsequent minor ANOVA analysis identifying the effects of all other genes (*hns* and *vimE* data excluded, lowercase letter groupings, same codification). We also analysed temperature/osmolarity (sucrose) effects in a similar one-way ANOVA: groups of the Tukey HSD analysis are defined by the greek letters α, β and γ (ANOVA by line, on the wild-type strain A4684).

nd, not determined.

Costechareyre, D., B. Dridi, Y. Rahbe, and G. Condemine. 2010. Cyt toxin expression reveals an inverse regulation of insect and plant virulence factors of *Dickeya dadantii*. *Environ Microbiol* 12: 3290-301.

A



pathogeny assays on aphid

Table 2. LT50 values for ingestion tests.

Strain	LT ₅₀ (days) ^a	P-value of survival analysis comparison (strain vs. wt) ^b
wt (A3952)	3.06 [2.11–4.45]	–
<i>Δtox</i> (A4977)	7.70 [5.26–11.28]	0.0003*
<i>hns</i> (A4112)	3.23 [2.28–4.59]	0.81
<i>vfmE</i> (A4111)	3.66 [2.59–5.21]	0.48
<i>pecS</i> (A3953)	NC	< 0.00001*
<i>phoP</i> (A4137)	5.00 [3.24–7.72]	0.16
<i>gacA</i> (A4239)	5.83 [4.49–7.06]	0.003*
<i>ompR</i> (A4567)	5.59 [3.76–8.31]	0.043*

a. Estimates of LT50 values for ingestion test were made using parametric survivor fit (Log-Normal distribution). Values are the time to reach 50% mortality of the initial aphid population, with lower and upper confidence intervals shown in brackets. NC: LT50 values not estimated, because the 50% mortality was not reached at 7 days after ingestion for *pecS* mutant. Results were obtained with 30 third instar aphid nymphs per treatment. The experiment was repeated twice with highly similar results, including survival analysis significance statistics (a two-way ANOVA identified no 'experiment' effect; see Fig. 2 caption).

b. P-value given in last column is that of the parametric survival comparison between wild-type strain and the respective mutants.

*Points to significant values at risk 0.05.

Table 3. LT50 values for injection tests.

Strain	LT ₅₀ (days) ^a	P-value of survival analysis comparison (strain vs. wt) ^b
wt (A3952)	1.14 [1.01–1.29]	–
<i>Δtox</i> (A4977)	1.20 [1.06–1.27]	0.54
<i>hns</i> (A4112)	1.31 [1.12–1.33]	0.028*
<i>vfmE</i> (A4111)	1.26 [1.12–1.42]	0.192
<i>pecS</i> (A3953)	0.95 [0.84–1.08]	0.041*
<i>phoP</i> (A4137)	1.12 [0.99–1.26]	0.78
<i>gacA</i> (A4239)	1.52 [1.34–1.72]	0.0003*
<i>ompR</i> (A4567)	1.07 [0.94–1.21]	0.408

a. Estimates of LT50 values for injection test were made using parametric survivor fit (Log-Normal distribution). Values are the time to reach 50% mortality of the initial aphid population, with lower and upper confidence intervals shown in brackets. Results were obtained with 30 third instar aphid nymphs per treatment. The experiment was repeated twice with highly similar results, including survival analysis significance statistics (a two-way ANOVA identified no 'experiment' effect; see Fig. 3 caption).

b. P-value given in last column is that of the parametric survival comparison between wild-type strain and the respective mutants.

*Points to significant values at risk 0.05.

Costechareyre, D., B. Dridi, Y. Rahbe, and G. Condemine. 2010. Cyt toxin expression reveals an inverse regulation of insect and plant virulence factors of *Dickeya dadantii*. *Environ Microbiol* 12: 3290–301.

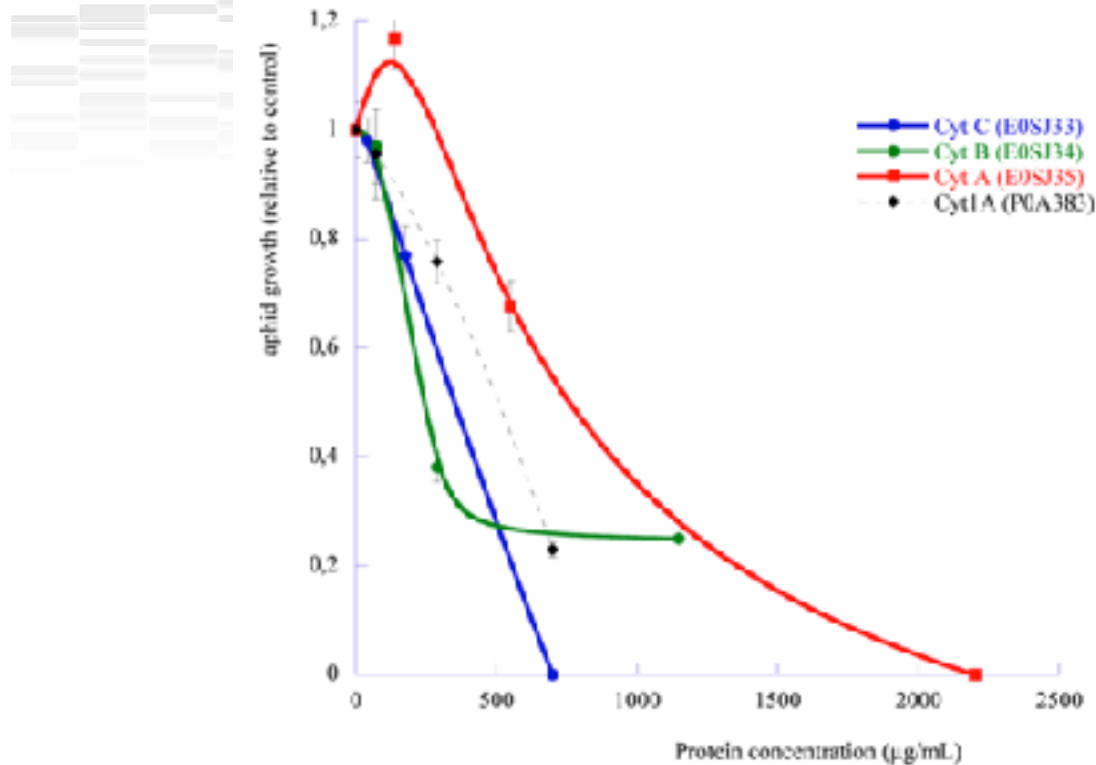
Cyt toxin expression reveals an inverse regulation of insect and plant virulence factors of *Dickeya dadantii*

- Their expression is induced at high temperature and at an **osmolarity** equivalent to that found in the **plant phloem sap**
- The regulators of cyt genes have also been identified: their expression is repressed by **H-NS** and **VfmE** and activated by **PecS**
- These genes are already known to regulate plant virulence factors, but in an **opposite way**
- Mutants of **other regulators** of plant virulence, GacA, OmpR and PhoP, that do not control Cyt toxin production, also showed reduced pathogenicity.

Costechareyre, D., B. Dridi, Y. Rahbe, and G. Condemine. 2010. Cyt toxin expression reveals an inverse regulation of insect and plant virulence factors of *Dickeya dadantii*. *Environ Microbiol* 12: 3290–301.

A

the cytABC are indeed aphid toxins...



Loth, K., D. Costechareyre, C. Landon, G. Effantin, Y. Rahbé, G. Condemine, and P. Da Silva. 2014. New Cyt-like δ -endotoxins from *Dickeya dadantii*: structure and aphicidal activity. *Sci. Reports* 2015 5:8791. doi: 10.1038/srep08791.

A

the structure of CytC has been solved by NMR

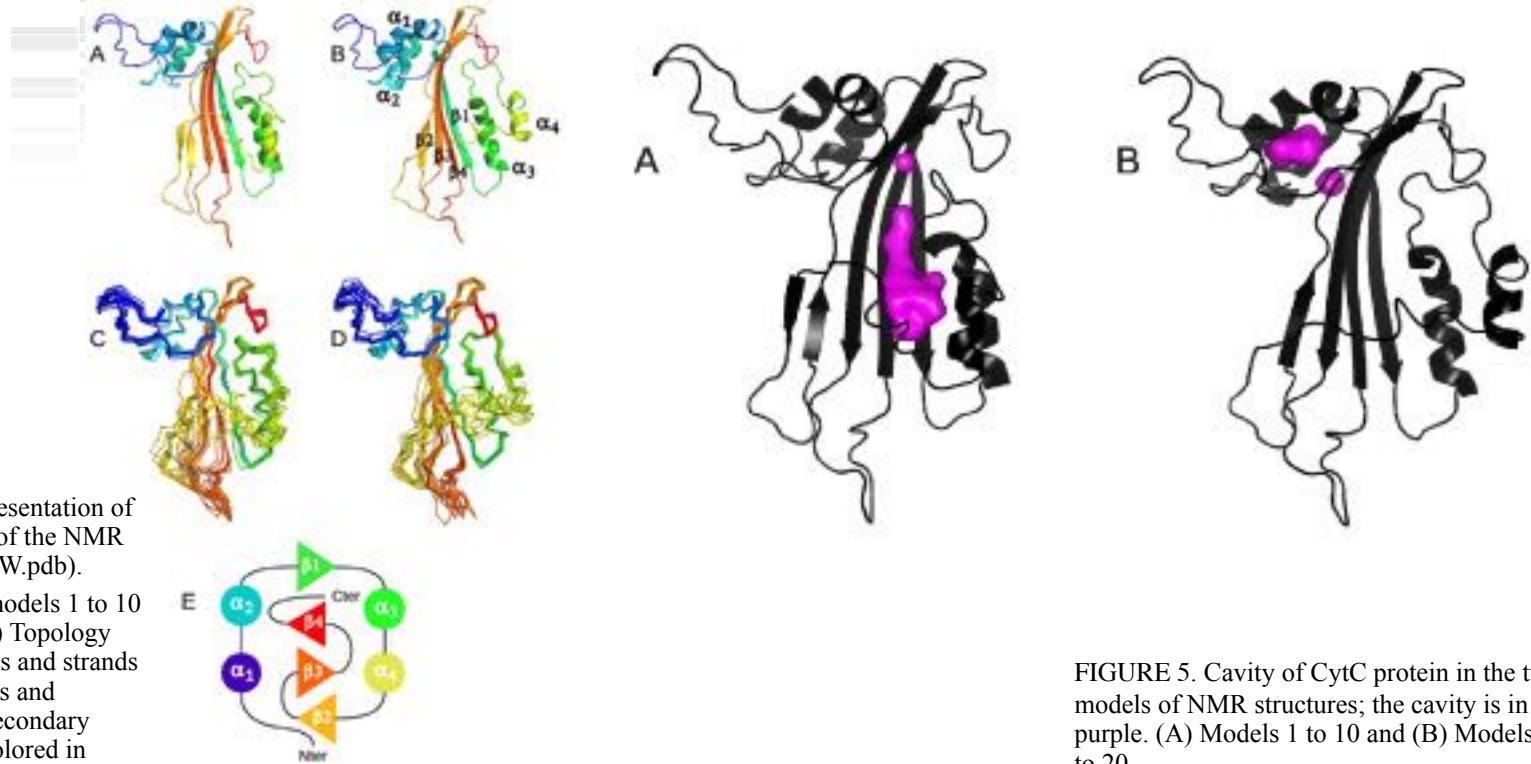


FIGURE 5. Cavity of CytC protein in the two models of NMR structures; the cavity is in purple. (A) Models 1 to 10 and (B) Models 11 to 20.

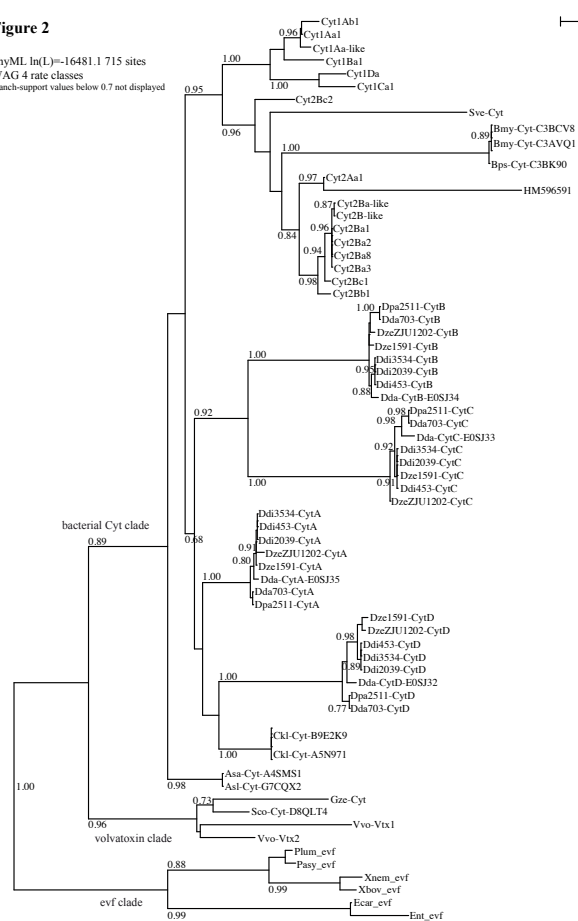
A



Cyt-like Phylogeny

Figure 2

PhyML ln(L)=-16481.1 715 sites
WAG 4 rate classes
branch-support values below 0.7 not displayed



Loth, K., D. Costechareyre, C. Landon, G. Effantin, Y. Rahbé, G. Condemine, and P. Da Silva. 2014. New Cyt-like δ -endotoxins from *Dickeya dadantii*: structure and aphicidal activity. *Sci. Reports* 2015 5:8791. doi: 10.1038/srep08791.

A

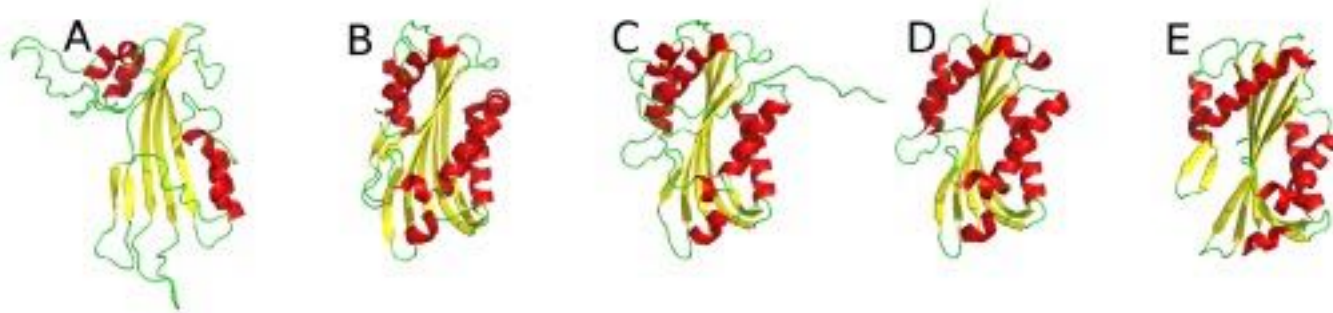


FIGURE 7. Ribbon representation of CytC (A), Cyt1Aa (B), Cyt2Aa (C), Cyt2Ba (D), and VVA2 (E). Helices are represented in red and β -strands in yellow.

Loth, K., D. Costechareyre, C. Landon, G. Effantin, Y. Rahbé, G. Condemine, and P. Da Silva. 2014. New Cyt-like δ -endotoxins from *Dickeya dadantii*: structure and aphicidal activity. *Sci. Reports* 2015 5:8791. doi: 10.1038/srep08791.

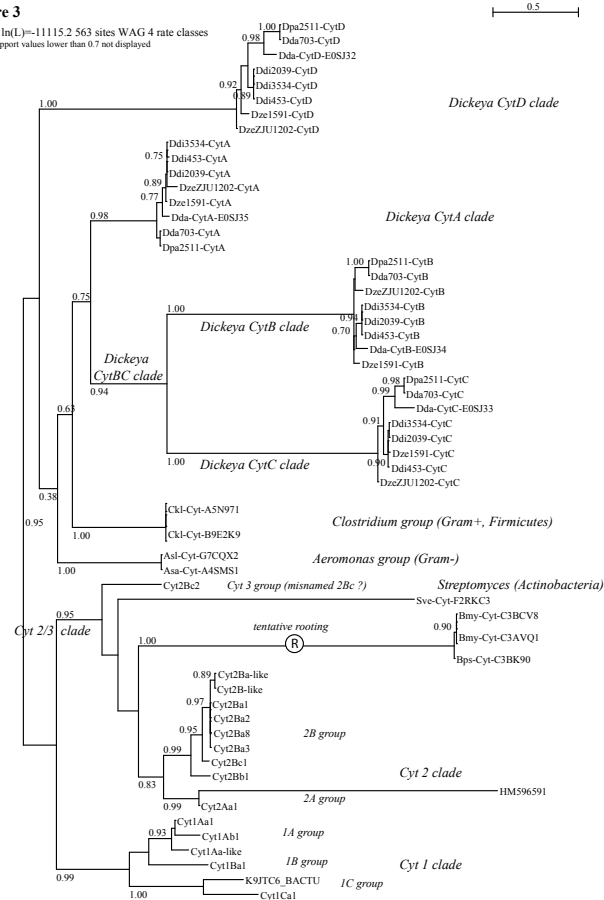
A

bacterial Cyt (ss) Phylogeny



Figure 3

PhyML ln(L) ~ 11115.2 563 sites WAG 4 rate classes
branch-support values lower than 0.7 not displayed



Loth, K., D. Costechareyre, C. Landon, G. Effantin, Y. Rahbé, G. Condemine, and P. Da Silva. 2014. New Cyt-like δ -endotoxins from *Dickeya dadantii*: structure and aphicidal activity. *Sci. Reports* 2015 5:8791. doi: 10.1038/srep08791.

A

other insect models for transient (multi-)pathogens

Table 1. Properties of bacterial pathogens

Pathogen	family	Genome size (Mb)	Plant hosts	Insect hosts	Nature of insect association	Plant pathogenicity factors	Insect pathogenicity factors
<i>Candidatus Liberibacter</i>	Rhizobiaceae	1.2	Citrus	Psyllid	Vector	–	–
<i>Dickeya dadantii</i>	Enterobacteriaceae	4.9	Potato, maize	Pea aphid	Host	<i>hpaA</i>	<i>cyt</i>
<i>Erwinia amylovora</i>	Enterobacteriaceae	3.8	Apple, pear	Pollinating insects	Vector	amylovoran, levan, <i>znpF</i> – <i>hsc</i>	–
<i>Erwinia aphidicola</i>	Enterobacteriaceae	~4	Bean, pea	Pea aphid	Host	–	–
<i>Erwinia tracheiptera</i>	Enterobacteriaceae	~4	Cucumber, melon	Cucumber beetle	Vector	–	–
<i>Pantoea stewartii</i>	Enterobacteriaceae	5.0	Maize	Fla beetle	Vector	<i>hpaWtr</i>	<i>zsa</i> (<i>zsaM</i>)
<i>Pectobacterium carolinianum</i>	Enterobacteriaceae	5.0	Potato	Aphid	Host	–	<i>znp1</i>
<i>Pseudomonas syringae</i>	Pseudomonadaceae	5.5	Bean	Pea aphid	Vector/host	<i>hpaHnc</i>	–
<i>Serratia marcescens</i>	Enterobacteriaceae	4.5	Pumpkin, squash	Squash bug	Vector	<i>oxyR</i>	–
<i>Xylella fastidiosa</i>	Xanthomonadaceae	2.1	Citrus, grape	Sharpshooter, spittlebug	Vector	<i>znpC</i>	<i>znpF</i>

- bacteria

- viruses

- aphid propagative phyto-viruses (reoviruses, rhabdoviruses, tospoviruses, tenuiviruses)

- honeybee and TRSV ? (tobacco ring spot virus)

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FEMS Microbiol Rev 88 (2011) 555–575



REVIEW ARTICLE

Insects as alternative hosts for phytopathogenic bacteria

Gautamchaly Nadarajah¹ & John Stavrinides¹

¹Department of Biology, University of Regina, Regina, Saskatchewan, Canada

A Adult alate pea aphids are significantly more resistant to (oral) infection by *Dda*

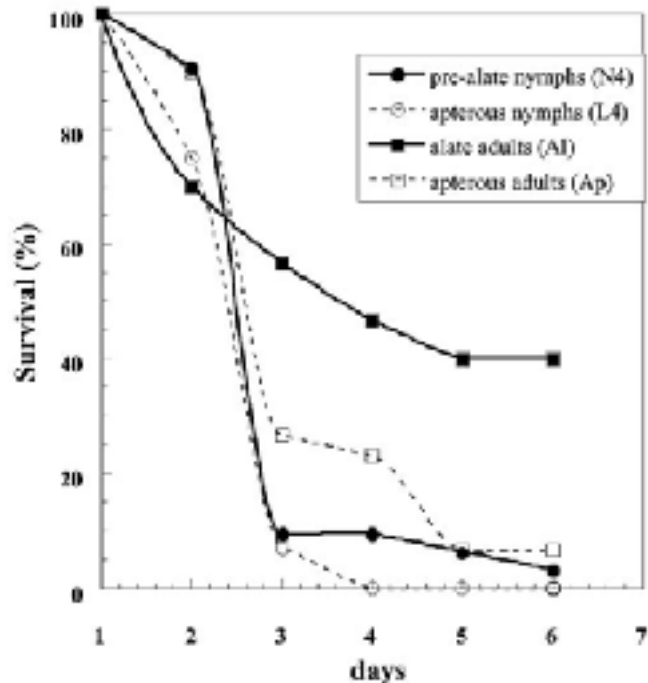
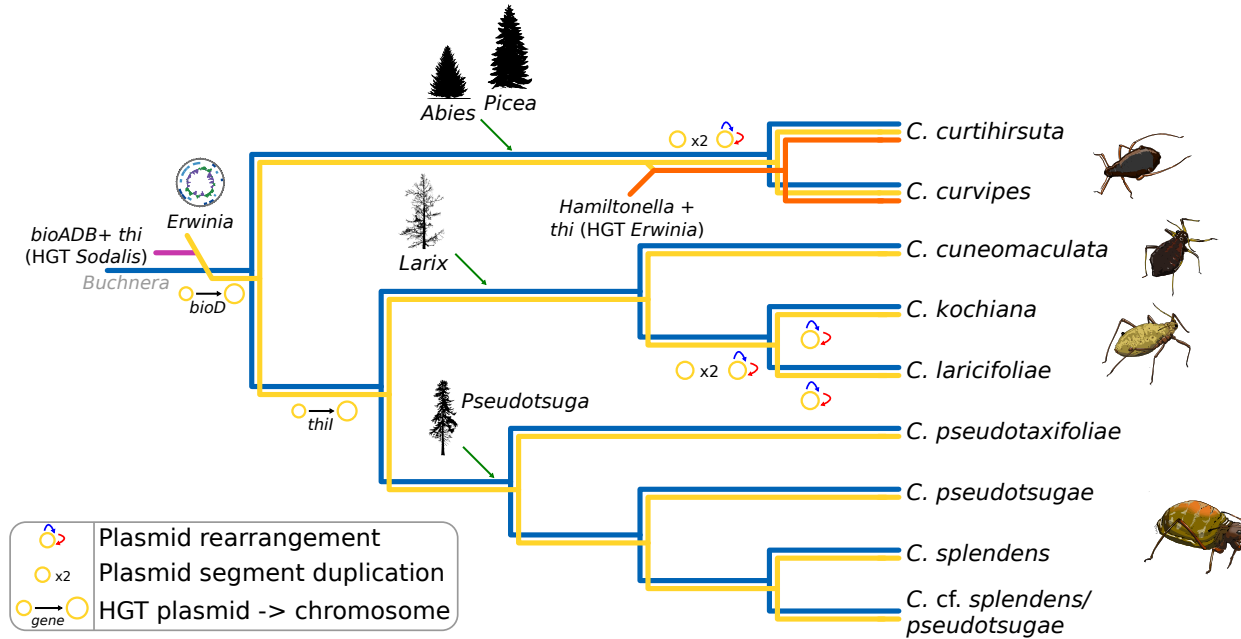


FIG. 3. Sensitivity of winged versus apterous developmental lineages of the pea aphid to *D. dadantii* oral infection at L4 and N4 nymphal stages and reproducing adult stages, 6 days after imaginal molt.

Grenier, A. M., G. Duport, S. Pages, G. Condemine, and Y. Rahbe. 2006. The Phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) Is a Pathogen of the Pea Aphid. *Appl. Environ. Microbiol.* 72: 1956-65.

A

A archetypal phytopathogenic bacterial clade turns in co-primary symbiont (*Erwinia haradaeae*, closest free-living relative *Erwinia pyrifoliae*)



A

Take home message 1

Complex evolutionary histories: frontiers between cognate symbioses, (multi-host) pathogenicity and vectors are sometimes tenuous

B

molecular mechanisms of transmission

Lessons from (plant) viruses to bacteria ?

A

Typologie de la vection virale (plantes)

	<i>NP</i> <i>(non persistent)</i>	<i>SP</i> <i>(semi-persistent)</i>	<i>P</i> <i>(persistent)</i> <i>= circulative</i>
<i>Persistence</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Circulation</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Multiplication</i>	<i>Non</i>	<i>Non</i>	<i>Rare</i>

A

Typologie de la vection virale (plantes)

	<i>NP</i> (<i>non persistent</i>)	<i>SP</i> (<i>semi-persistent</i>)	<i>P</i> (<i>persistent</i>) = <i>circulative</i>
<i>Persistence</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Circulation</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Multiplication</i>	<i>Non</i>	<i>Non</i>	<i>Rare</i>

A

Typologie de la vection virale (plantes)

	<i>NP</i> (<i>non persistent</i>)	<i>SP</i> (<i>semi-persistent</i>)	<i>P</i> (<i>persistent</i>) = <i>circulative</i>
<i>Persistence</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Circulation</i>	<i>Non</i>	<i>Non</i>	<i>Oui</i>
<i>Multiplication</i>	<i>Non</i>	<i>Non</i>	<i>Rare</i>

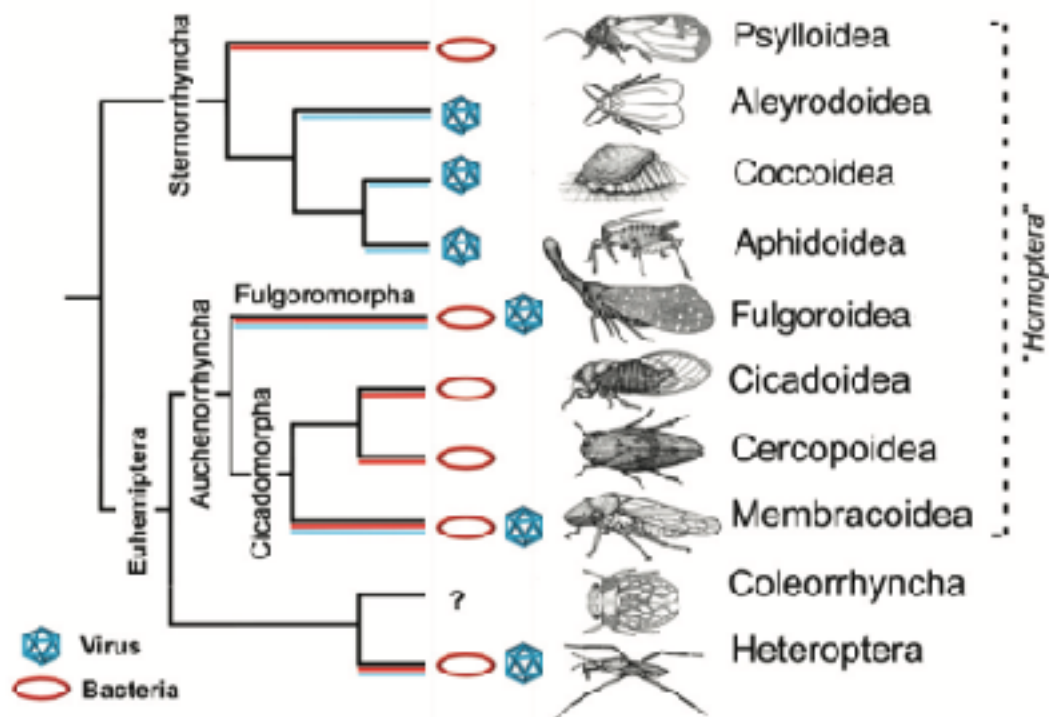
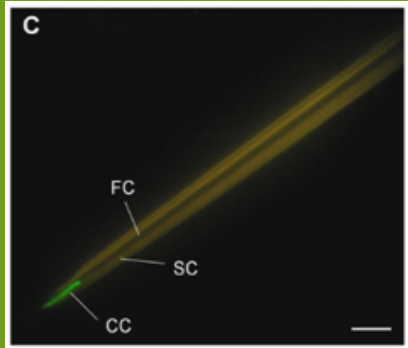


FIGURE 1 | Hemiptera taxa: reported vectors and groups of plant pathogens. Specific plant pathogens are listed in Supplementary Table S1. Branches where species have been reported as transmitting virus are labeled in blue, while those transmitting bacteria are labeled in red. Figure modified with permission from Gullan and Cranston (2014).

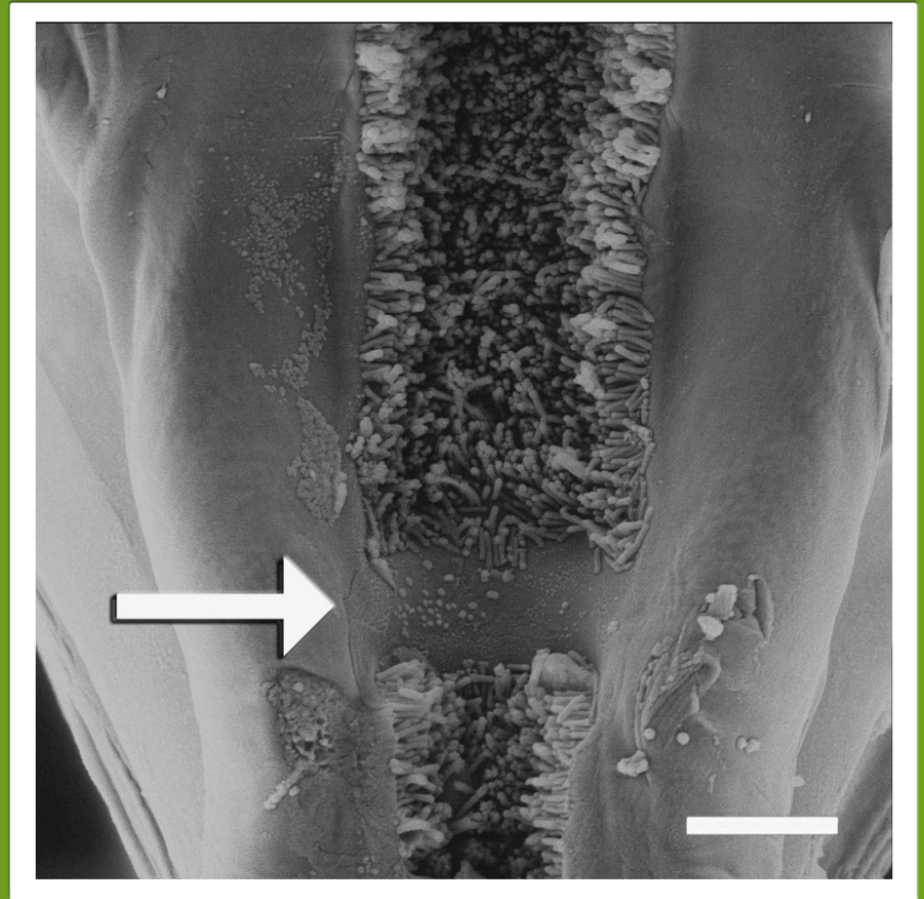
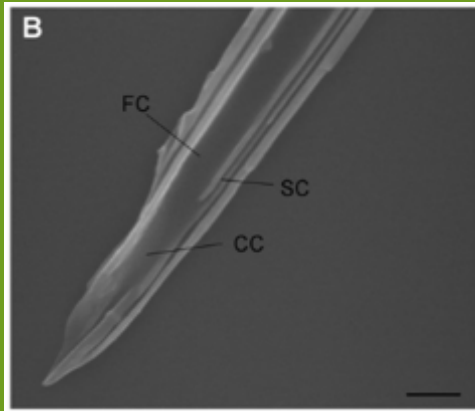
Class Family	Pathogen	Genome size (Mb)	Plant tissue tropism	Plant host	Vectors	Location/ Insect organs	Reference
Gammaproteobacteria <i>Xanthomonadaceae</i>	<i>Xylella fastidiosa</i>	2.7	Xylem (a)	Wide host range	<i>Homalodisca vitripennis</i> , <i>Graphocephala atropunctata</i> (+ Others)	Non-Circulative/ Cybarium, foregut	Backus and Morgan, 2011
	FimA, FimB, HxfA, HxfB, O-antigen						
Mollicutes <i>Spiroplasma</i> family	<i>Spiroplasma citri</i>	1.6	Phloem (a)	Citrus	<i>Circulifer tenellus</i>	Circulative/	Fletcher et al., 1998
	<i>Spiroplasma kunkelii</i>			Corn	<i>Dalbulus maidis</i>	Hemolymph bacteriocyte, salivary glands	
	pili and membrane proteins (spiralins...)						
Mollicutes <i>Acholeplasmataceae</i>	"Candidate <i>Phytoplasma</i> spp."	0.6	Phloem (b)	Wide host range: Asteraceae horticulture crops	<i>Macrostelus duschlingae</i> (+ Others)	Circulative/ Hemolymph bacteriocyte, salivary glands	Beanland et al., 2000
	P38, VmpA and secreted proteins						
Alphaproteobacteria <i>Rhizobiaceae</i>	"Candidate <i>Liberibacter</i> spp."	1.2	Phloem (b)	Citrus Solanaceae Apiaceae	<i>Diuraphis citri</i> , <i>Bactericera cockerelli</i> , <i>Bactericera trigonica</i> , <i>Trioza apicalis</i>	Circulative/ Hemolymph bacteriocyte, salivary glands	Ammar et al., 2011
	membrane protein (lasAI autoptransporter)						

The bacterial group is routinely culturable **(a)** or non-culturable **(b)**.



aphid / virus

Et du côté insecte ?



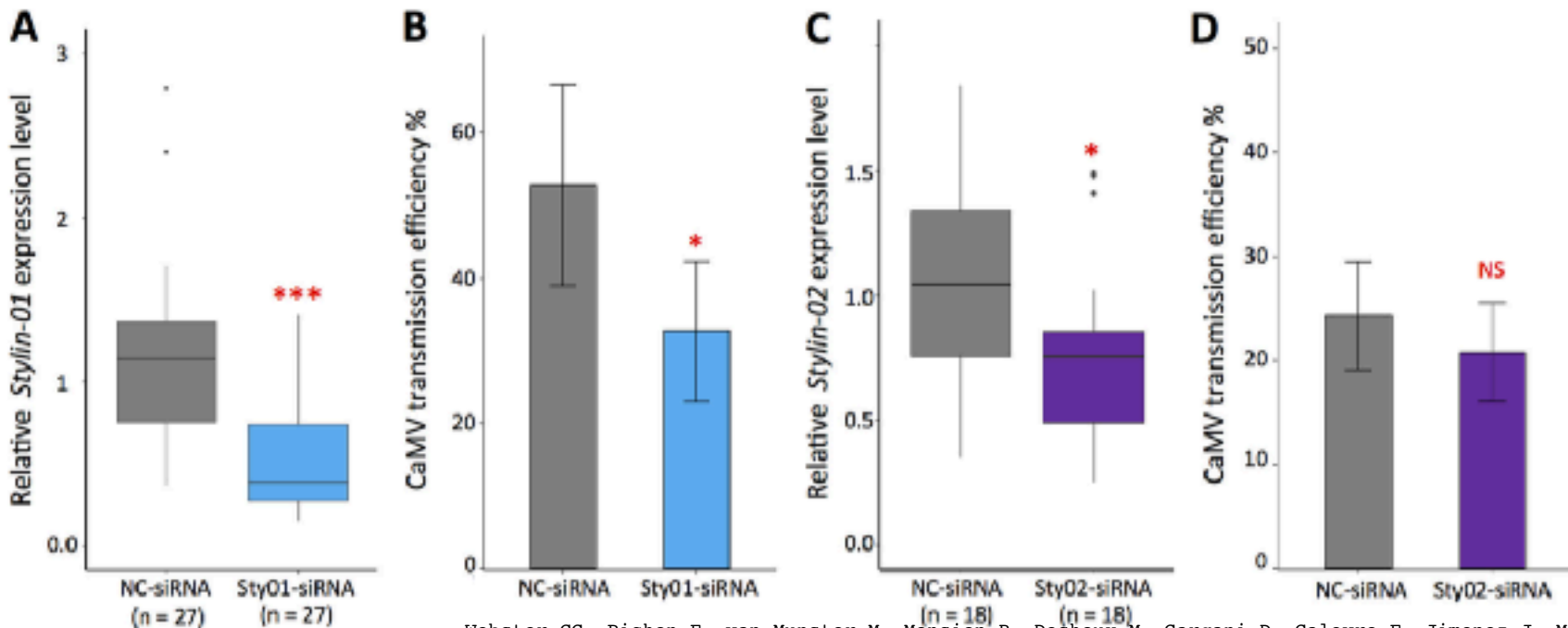
CaMV - cauliflower mosaic

Xylella fastidiosa - Pierce disease

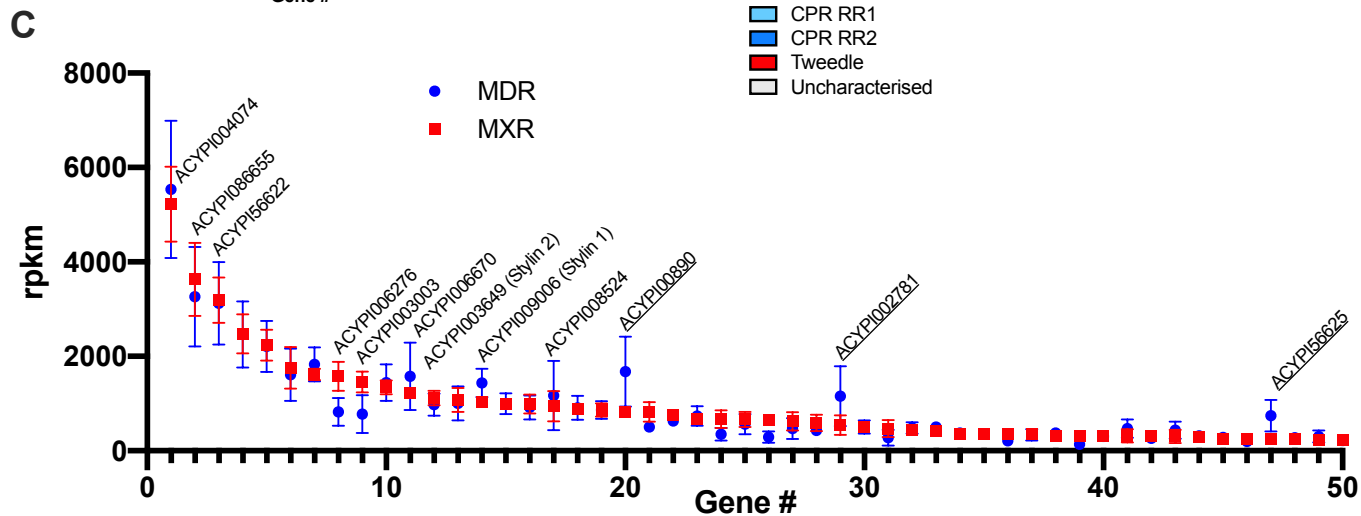
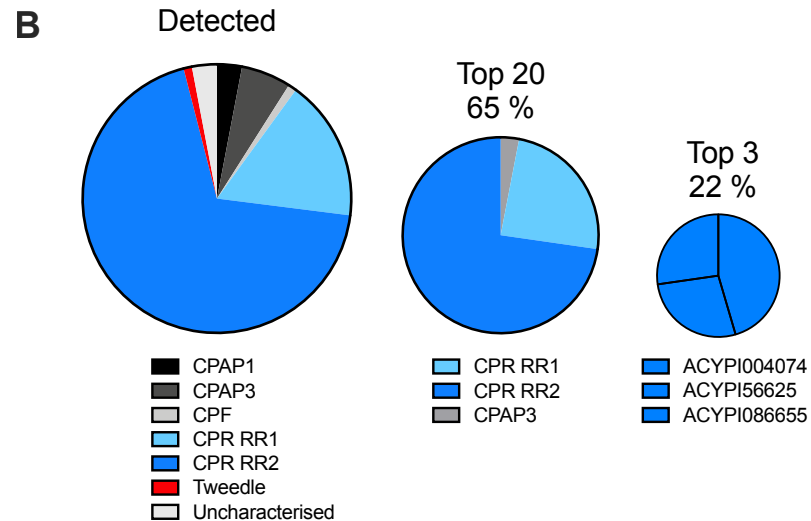
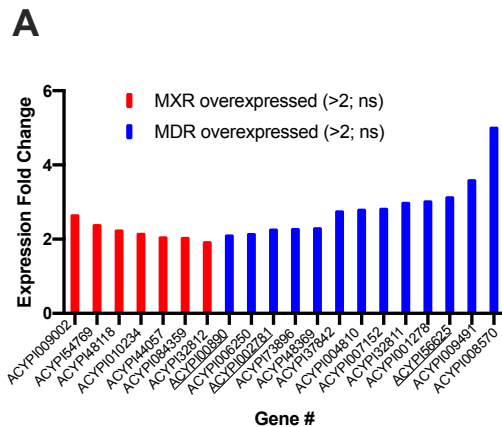
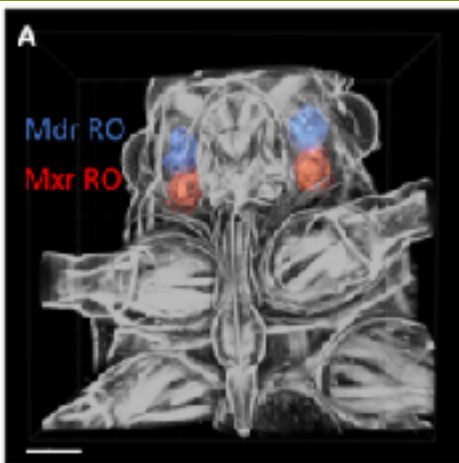
Stylin 1 is the (P2) CaMV receptor

Plant Virus Receptor in Aphid Stylets

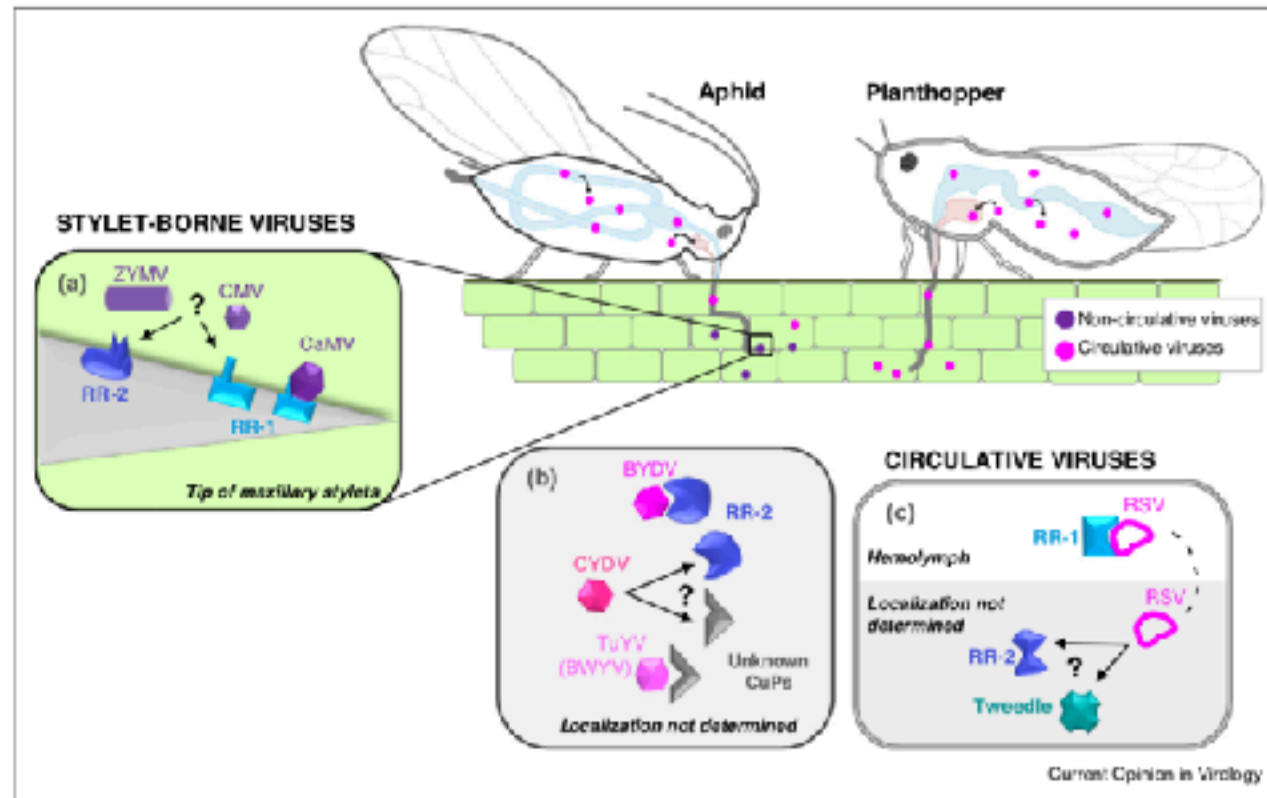
Journal of Virology



Webster CG, Pichon E, van Munster M, Monsion B, Deshoux M, Gargani D, Calevro F, Jimenez J, Moreno A, Krenz B, Thompson JR, Perry KL, Fereres A, Blanc S, Uzest M. Identification of Plant Virus Receptor Candidates in the Stylets of Their Aphid Vectors. *J Virol.* 2018 Jun 29;92(14)

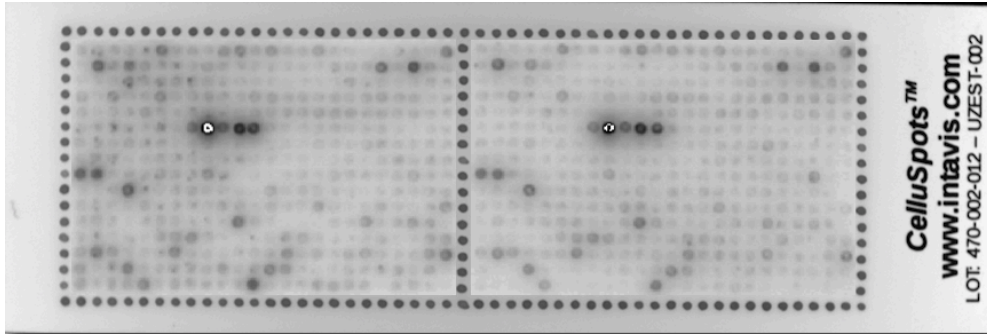


Guschinskaya N, Ressnikoff D, Arafah K, Voisin S, Bulet P, Uzest M, Rahbé Y: Insect mouthpart transcriptome unveils extension of cuticular protein repertoire and complex organization. iScience 2019, in revision.



Plant virus–cuticular protein (CuP) interactions. **(a)** Interaction of noncirculative viruses with cuticular proteins at the tip of aphid maxillary stylets: cauliflower mosaic virus (CaMV) with RR-1 protein via its helper protein P2 [15**], cucumber mosaic virus (CMV) with RR-1 and/or RR-2 proteins [22,25], zucchini yellow mosaic virus (ZYMV) via its helper protein HC-Pro with RR-1 and/or RR-2 proteins [17]. **(b)** Interaction of barley yellow dwarf virus (BYDV), cereal yellow dwarf virus (CYDV) and turnip yellow virus (TuYV), three circulative viruses, with RR-2 and/or unknown cuticular proteins within their aphid-vector body [16,18**,20]. **(c)** The nucleocapsid protein of the circulative rice stripe virus (RSV) binds an RR-1 protein in the hemolymph of its planthopper vector [15**], and may also interact with another RR-2 and a Tweedle cuticular protein [26].

Deshoux, M., Monsion, B. & Uzeyt, M. Insect cuticular proteins and their role in transmission of phytoviruses. *Curr Opin Virol* 33, 137–143 (2018).



→ 50 peptide sequences correspond to 50 positive spots

→ belong to 23 CuPs

→ For example: 3 peptides sequences (326, 324, 325) from CuP ACYPI010088

No de spot	Int-Moy Corr	seq peptidique	ACYPI
326	97,8	YGFPGTAVPYGYGPTAVP	ACYPI010088
293	94,4	DPYPAEDLRPKNVGVKTQ	ACYPI004305
193	88,2	GSFYAFDFGNNNGGAS	ACYPI006276
308	85,5	PVRYAFSGSKPLKNTLAV	ACYPI008524
244	82,0	MTEPPTFYQPAKPVAKR	ACYPI002781
349	78,9	RYADSSKSMSSRANNYYG	ACYPI44057
227	78,1	QNAEDYNDQSAVVSARKS	ACYPI002781
350	75,2	YYGVQYYAPPVVHGTAA	ACYPI44057
314	73,9	SYSAPSYSAPSYSAPSYS	ACYPI009152
6	68,5	ENGYQPYGAHLPTPPI	ACYPI009006
194	68,2	YAFDFGNNNGGASPEY	ACYPI006276
245	62,8	AKRTRPAAQLSTNYRTA	ACYPI002781
267	62,5	APAGQHHTVVPILKQVNR	ACYPI000890
205	59,7	SDGGKSAAGVVKKVLVSK	ACYPI37842
203	59,6	DPTAASVQTTTGRNRKK	ACYPI37842
361	59,3	RRPSPVNDVQRRRQQQ	ACYPI51165
184	58,2	APAYVFPSPSGRSLAKNR	ACYPI006276
224	53,9	LSTSPRYDPRISERSTT	ACYPI37842
28	53,3	AYSIVDPNGALQQYKYS	ACYPI009491
11	53,2	QQYIGGPQQQFIGGPNFA	ACYPI002877
21	51,1	AVAQQVAQAKLEPPQFNK	ACYPI002877 & ACYPI000308
201	50,6	DVGRDKRVSYSASNSGPF	ACYPI37842
163	47,3	GSYSYVDPNGQRRTVSYT	ACYPI006250
135	44,0	PVYKNVDNKLPAIVRQSQ	ACYPI001775
365	42,5	NLNDRGRITTLGDKQPSV	ACYPI51165
78	42,2	PTTPSSPSSSYQGNFGSY	ACYPI008113
20	40,8	GVHPAIRAQAQQVAQAK	ACYPI002877 & ACYPI000308
26	40,6	QNEFGQYSYGYNDGFSK	ACYPI009491
324	40,1	PVAEPLSYSAAYLPPKPM	ACYPI010088
49	39,3	DPLKQKQPTLNSSPAKNS	ACYPI005591
142	38,0	SLELLIASQPQKPEDSKKK	ACYPI001775
10	37,6	SPYFNGFRNGAYPQQYI	ACYPI002877 & ACYPI000308
337	36,4	IEIIPASAWSTFTGPVS	ACYPI38155
280	36,3	QDNASPFPGNTESNSIQRR	ACYPI001681
24	35,7	SLYFSGPQYYSGYAAPA	ACYPI009491
75	34,7	SIEPQQMMVYVNNQQGRY	ACYPI005591
8	34,5	ESLRYLASLPSTPEPKYQ	ACYPI009006
304	33,6	EAKVVATAPAKIVTPTRT	ACYPI008524
83	33,6	QSGVSSYQSTPASLQYSK	ACYPI008113
315	32,9	SSYSSSSYSSSYSTAAP	ACYPI009152
271	32,4	APVAVRQQQWFSAGPQQK	ACYPI000890
102	32,4	DGSYSYSYETGNGISVSE	ACYPI008113
374	32,2	KTISKARGQPLEPKVYTM	Pep-M2.2
52	31,2	YNFKYETGNGIFAEERGV	ACYPI005591
77	30,9	HPQGYESEASSGYGSPTT	ACYPI008113
234	30,7	DEEPAAPVVKRPSPAKP	ACYPI002781
325	30,4	KPMVVGGPLHYNSPYDG	ACYPI010088
309	30,3	KPLKNTLAVPETKTKTGY	ACYPI008524
229	30,1	HNEDEGSYTYGVEGSDGSF	ACYPI002781
148	30,1	YQVEYFADADNGFVAKGA	ACYPI066082

Webster CG, Thillier M, Pirolles E, Cayrol B, Blanc S, Uzest M. Proteomic composition of the acrostyle: Novel approaches to identify cuticular proteins involved in virus-insect interactions. Insect Sci. 2017 Dec;24(6):990-1002

A

Take home message 2

Cuticle is more than chitin

A biological solid is more than a surface

Cuticular proteins carry biologically relevant surface information

Insects (& microbes, & plants) are prone to climate stress

Lessons (?) from plant viruses

1. Berges, S. E. et al. Interactions Between Drought and Plant Genotype Change Epidemiological Traits of Cauliflower mosaic virus. *Front. Plant Sci.* 9, 703 (2018).
2. Yvon, M., Vile, D., Brault, V., Blanc, S. & van Munster, M. Drought reduces transmission of Turnip yellows virus, an insect-vector-borne circulative virus. *Virus Res* 241, 131–136 (2017).
3. van Munster, M. et al. Water deficit enhances the transmission of plant viruses by insect vectors. *PLoS ONE* 12, e0174398 (2017).

CaMV and TuMV on turnip

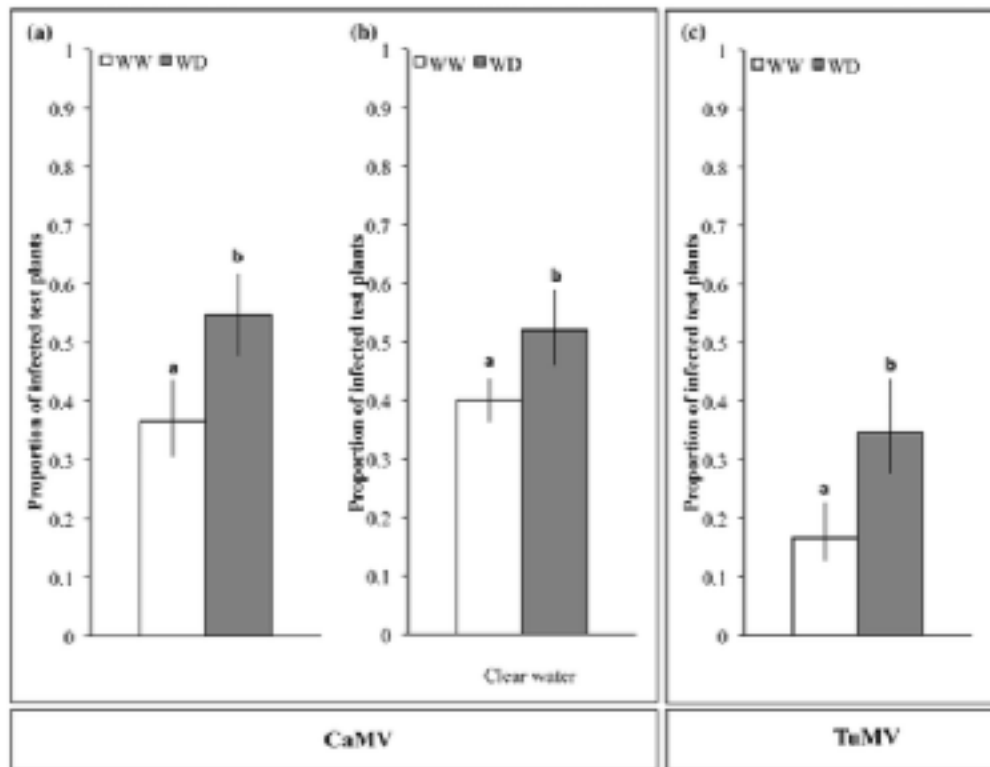


Fig 2. Aphid-transmission of CaMV and TuMV from turnip plants grown under Well-Watered (WW) or Water Deficit (WD) conditions. (a-c) Means of the proportion of infected test plants calculated from a pool of three independent experiments for each viral species (a, c) and two additional experiments conducted with CaMV-infected plants (b). In each experiment and for each condition, $n = 10$ source plants and $n = 12$ aphids per source plants individually transferred onto $n = 12$ receiving test plants. Watering was performed with a nutrient solution (a, c) or clear water (b). Error bars represent 95% confidence intervals and different lowercase letters indicate significant differences between water treatments according to a χ^2 test on the likelihood ratios ($p < 0.05$) for both CaMV and TuMV.

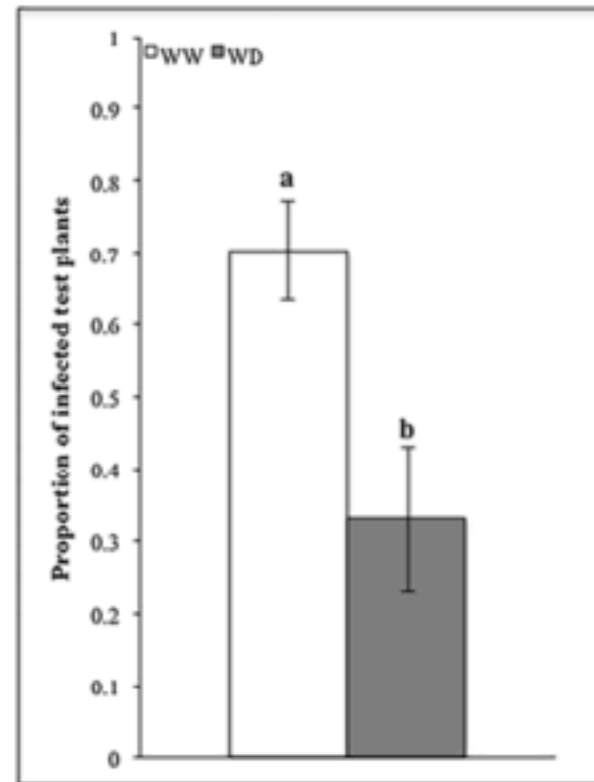
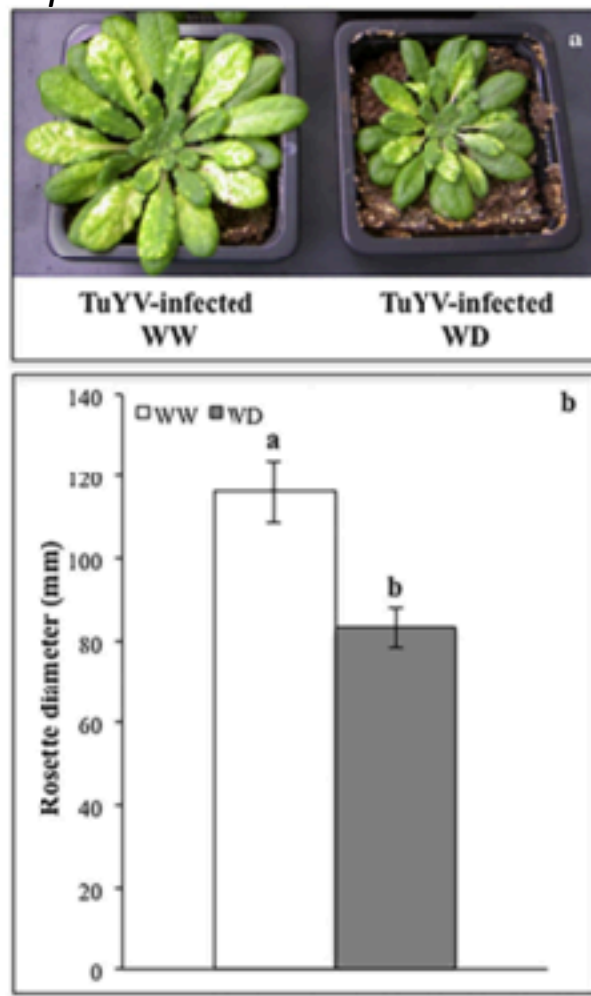
Mutant TuMV on *Arabidopsis*

Fig. 2. Aphid transmission of TuYV-SUL from *A. thaliana* plants grown under well-watered (WW) or water deficit (WD) conditions as virus source. Histograms indicate the average percentage of infected test plants from three independent experiments. Bars represent standard error (SEM) and different lowercase letters indicate significant differences between water treatments according to a χ^2 test on the likelihood ratios ($p \leq 0.05$).

C

Transmission & climate change

Take home message 3

Versatile outcomes

Collaborations

Dickeya, (also) an insect pathogen

Groupe Lyon BF21

Groupe Lyon MAP

Pedro Da Silva

Guy Condemine
(Denis Costechareyre)
(Bedis Dridi)
Géraldine Effantin

Natalia Guschinskaya



Cuticle microbe interactions

Groupe Montpellier CIRAD INRA BGPI

Marilyne Uzest
Manuella van Munster
Stéphane Blanc

Collaborations

Dickeya, (also) an insect pathogen

Groupe Lyon BF21

Groupe Lyon MAP

Pedro Da Silva

Guy Condemine
(Denis Costechareyre)
(Bedis Dridi)
Géraldine Effantin

Natalia Guschinskaya



Cuticle microbe interactions

Groupe Montpellier CIRAD INRA BGPI

Marilyne Uzest
Manuella van Munster
Stéphane Blanc

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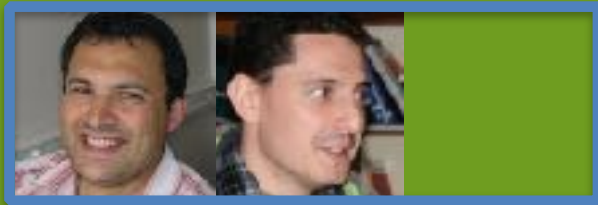
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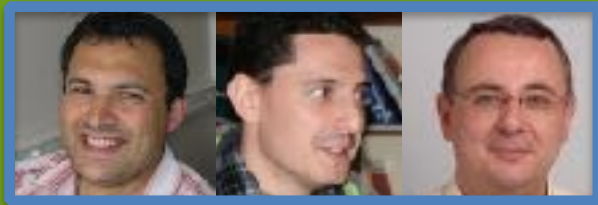
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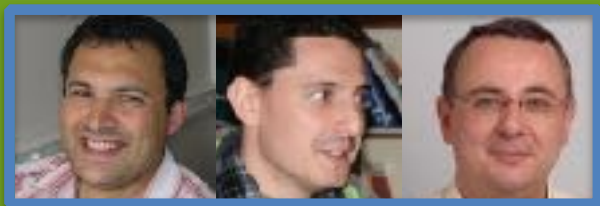
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