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Detection and quantification of cereal geminiviruses

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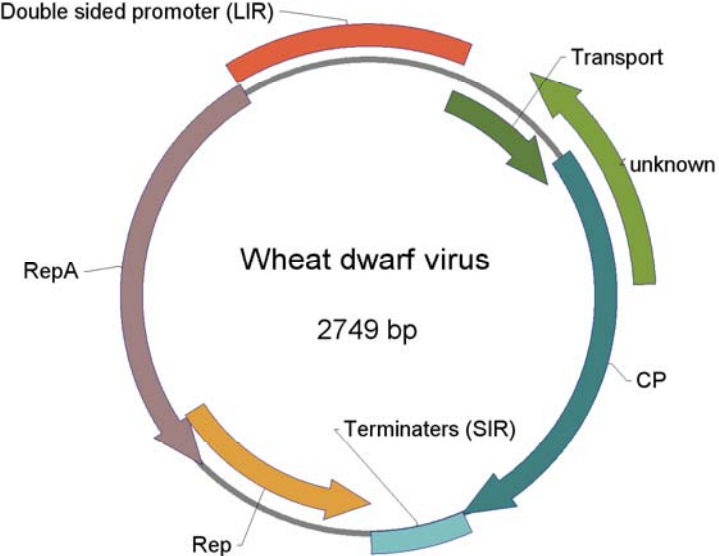
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Cereal geminiviruses

Wheat dwarf virus, Barley dwarf virus,
Oat dwarf virus,
(WDV, BDV, ODV),
transmitted by the leafhopper
Psammotettix alienus Dahlb. in a persistent manner



Alain Ramel

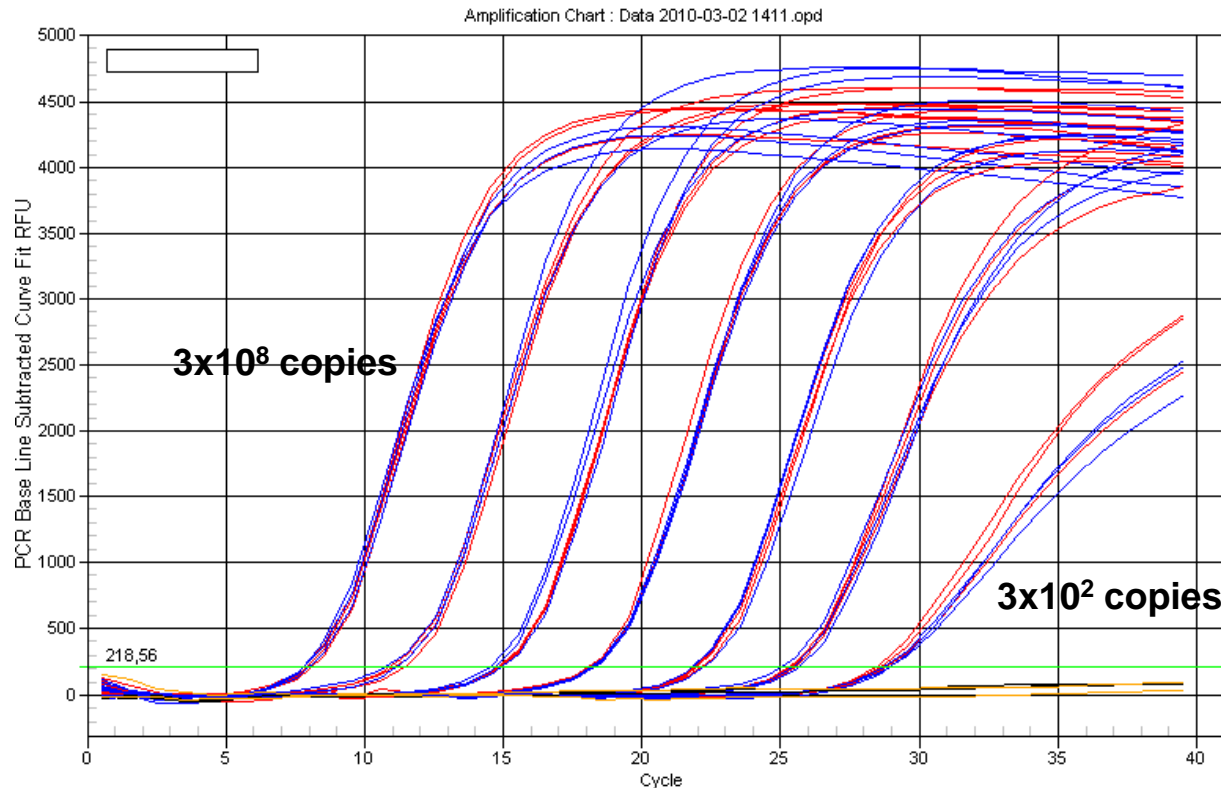


photo: Jörg Schubert



Nature Precedings : doi:10.1038/npre.2011.5735.1 : Posted 28 Feb 2011

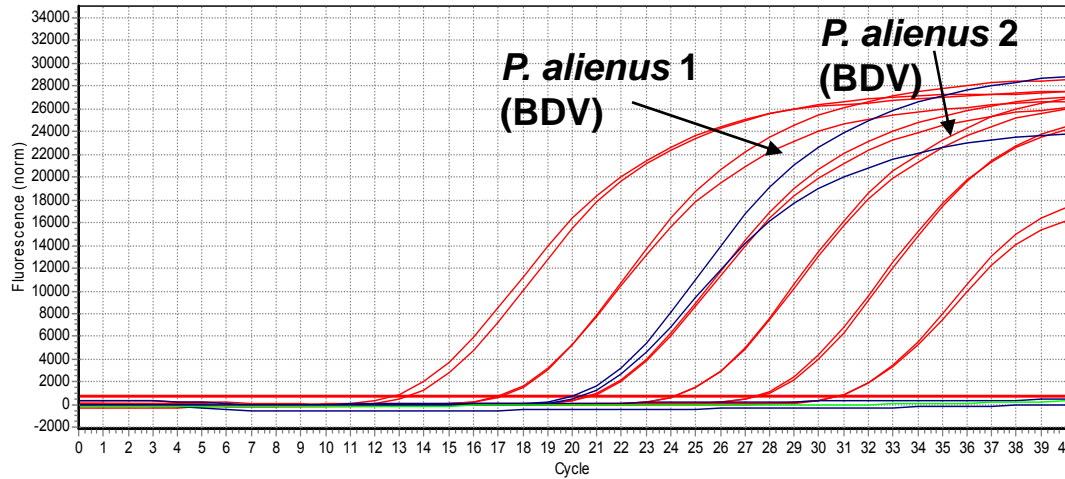
Detection with qPCR: absolute quantification



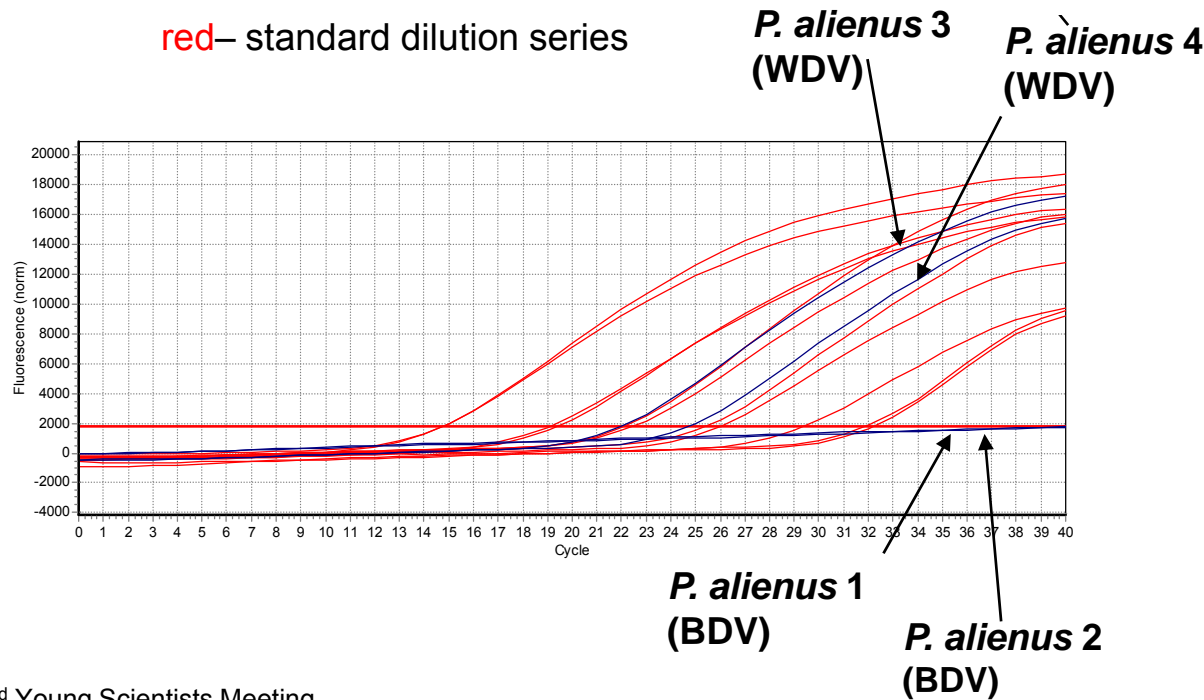
qPCR with SybrGreen, WDV-standard dilution series: 3×10^8 to 3×10^2 copies
blue: plasmid standard mixed with negative plant DNA (30 ng), red: plasmid standard without plant DNA, yellow: NTC, black: negative plant DNA.



TaqMan-Probes - differentiation between BDV and WDV



BDV-specific FAM-probe (λ_{\max} 520nm)



WDV-specific Yakima Yellow-probe (λ_{\max} 549nm)



Comparison of sensitivity of DAS-ELISA and qPCR



	ELISA					PCR				
	A	B	C	D	E	A	B	C	D	E
dilution										
pure sap	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
1:10	Red	Red	Red	Red	Red					
1:100	Red	Red	Red	Red	Red					
1:1000	Red	Red	Red	Red	Red					
1:10000	Yellow	Yellow	Yellow	Yellow	Red	Red	Red	Red	Red	Red
1:100000	Green	Green	Green	Green	Yellow	Red	Red	Red	Red	Red
1:1000000	Green	Green	Green	Green	Green	Red	Red	Red	Red	Red
1:10000000	Green	Green	Green	Green	Green	Red	Red	Red	Red	Red
1:100000000	White	White	White	White	White	Red	Red	Red	Red	Red
negative plant	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green

WDV- positive Greenhouse plants (A-E), 8 weeks p. i.,
sap diluted with sap of WDV-negative plants

Red	positive
Yellow	positive past 2h
Green	negative
White	not tested

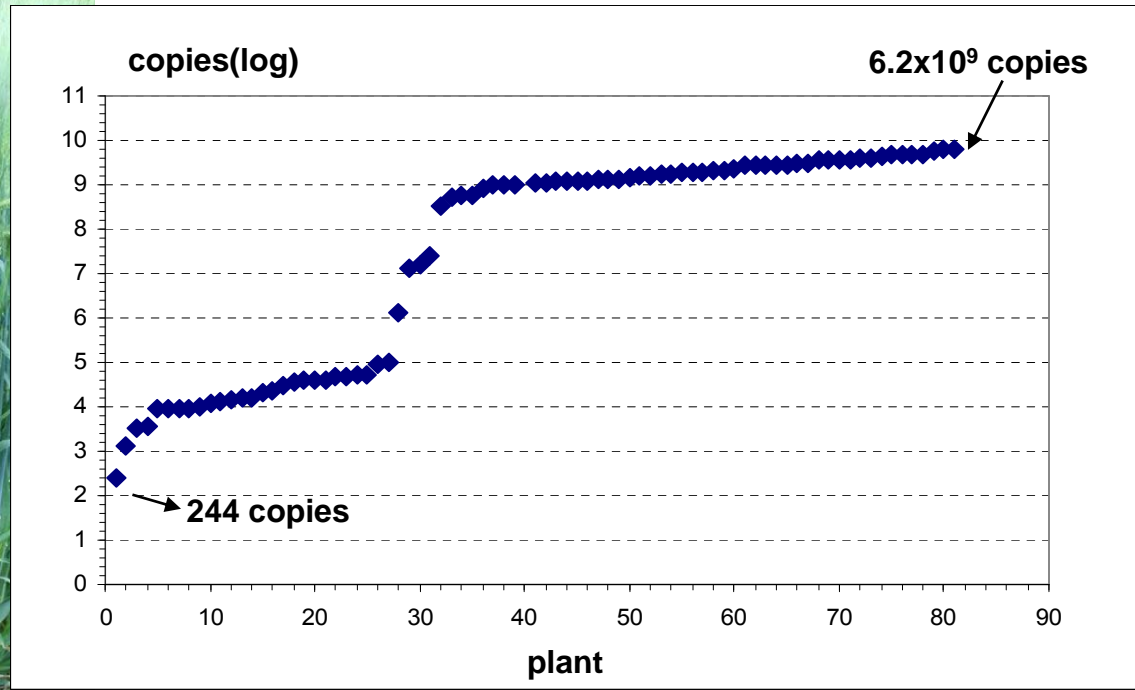


Field plants



photo: Nadine Drechsler

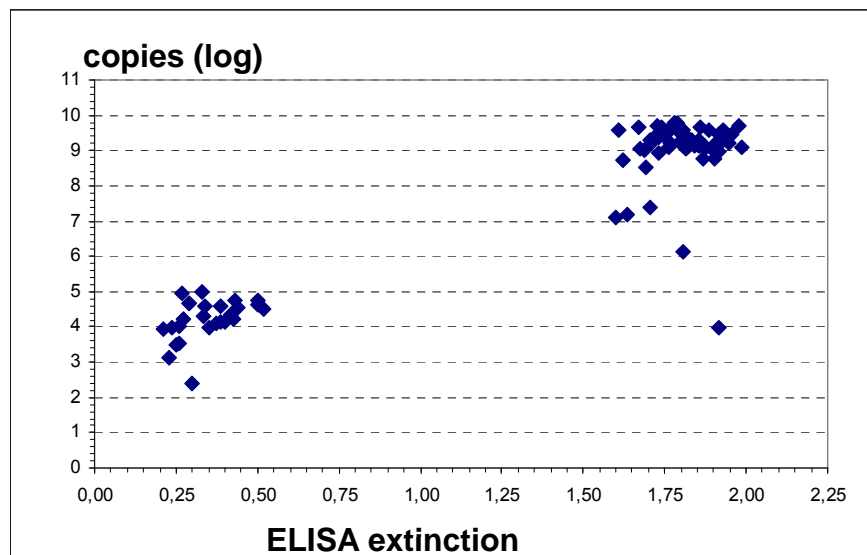
winter barley, early June



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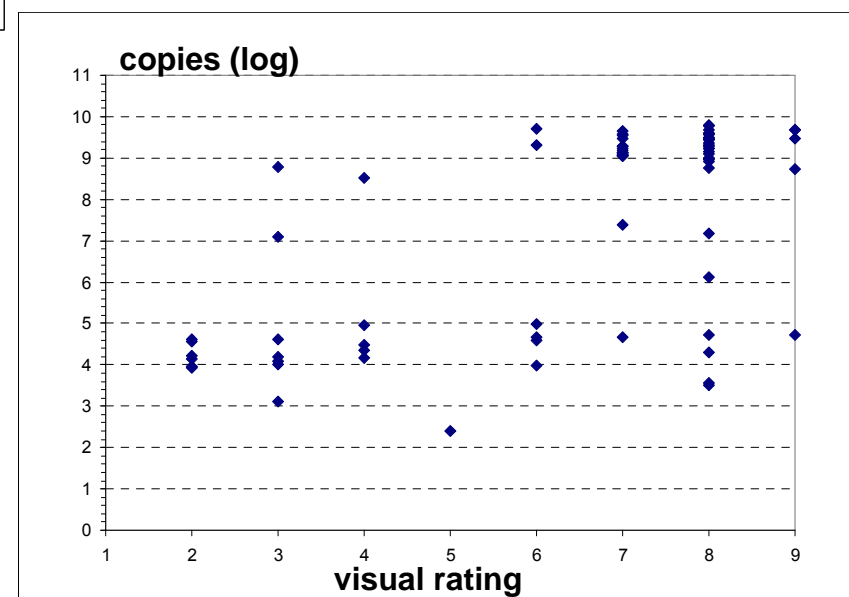


Field plants

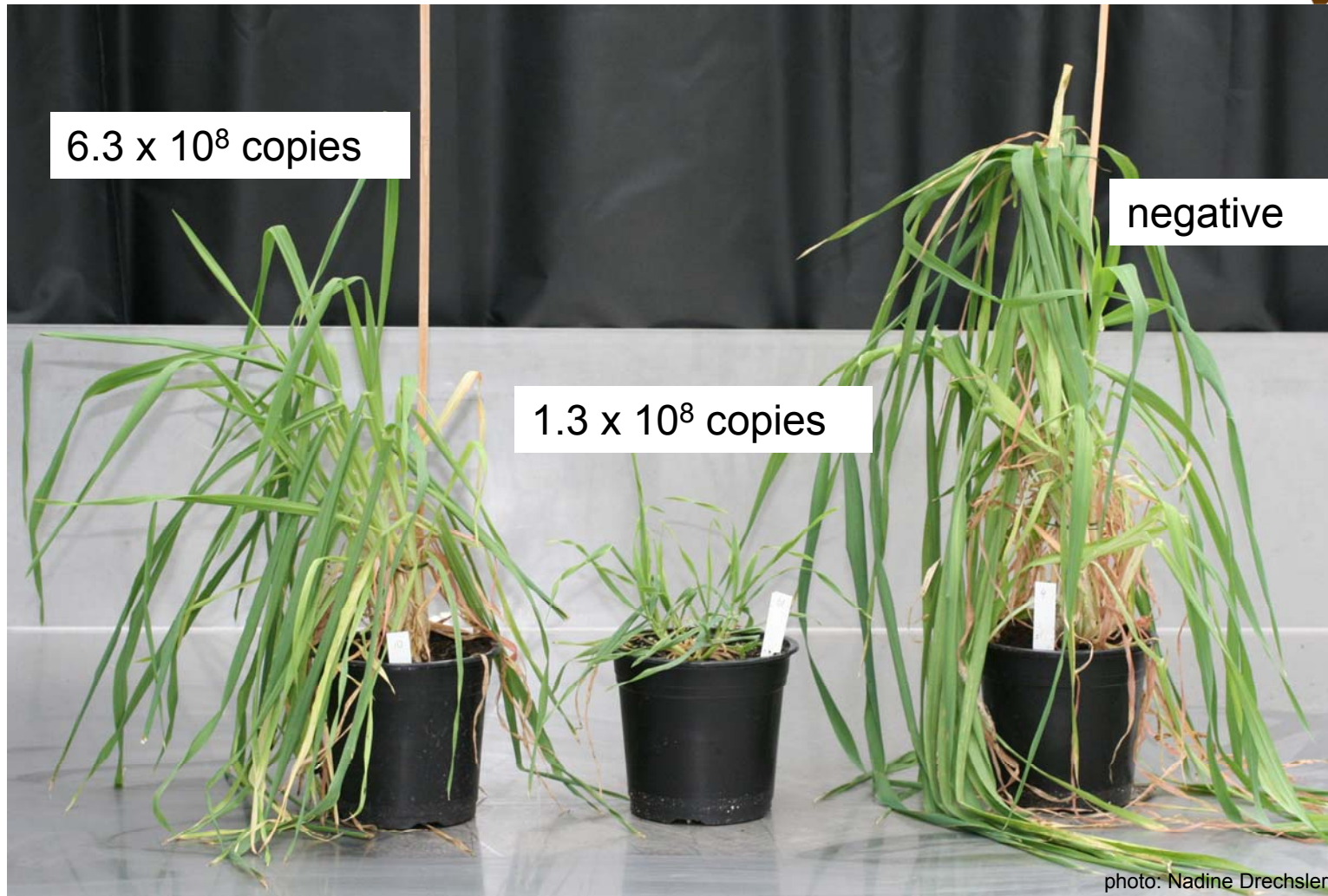


Pearson correlation: 0.932

Spearman correlation: 0.592

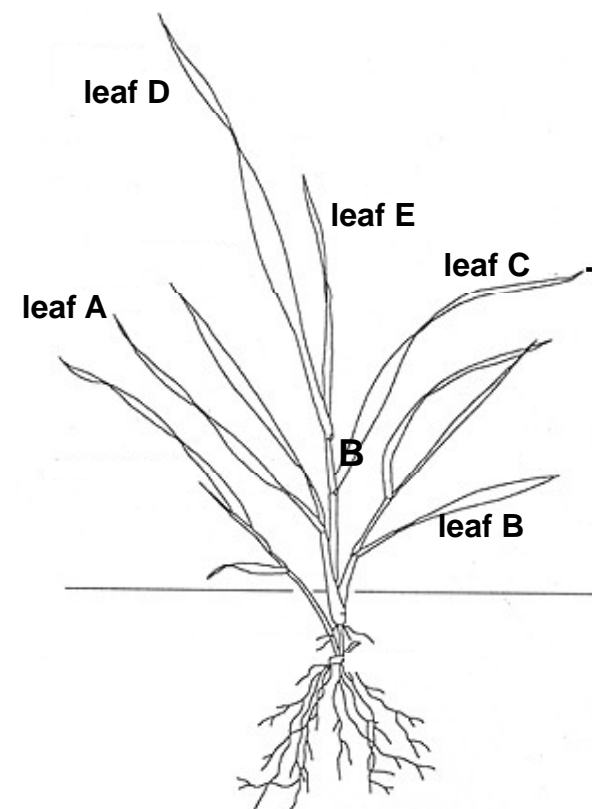
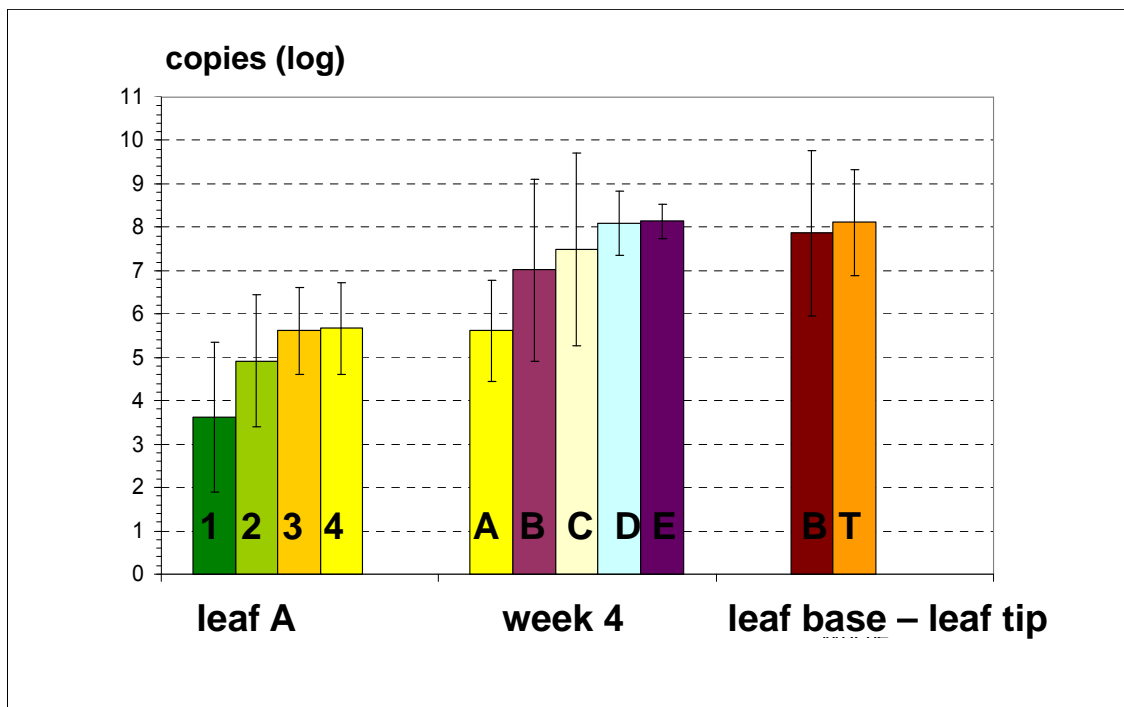


Virus titer and symptom expression



Rubina, 16 weeks past inoculation with *Psammotettix alienus*

Distribution of WDV in the plant

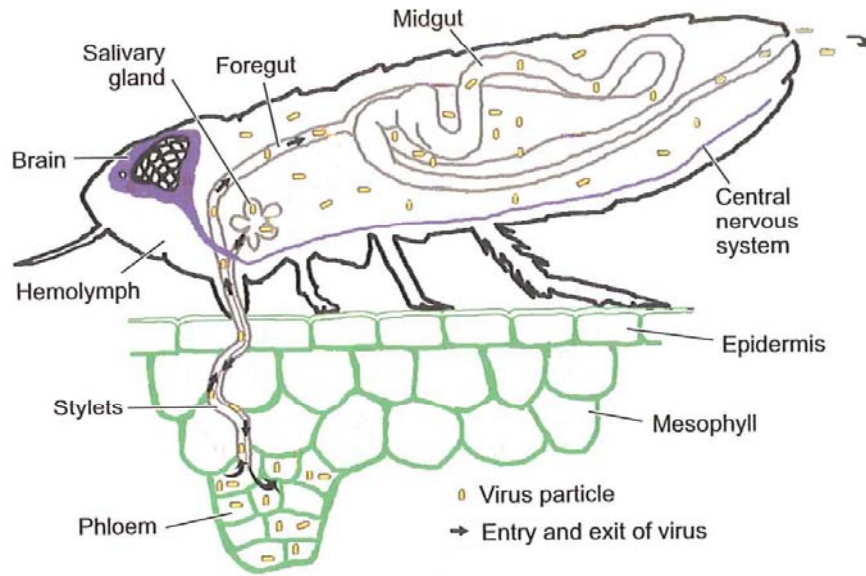


Winter barley ,Rubina‘, grown under greenhouse conditions, inoculated with 3 leafhoppers for 1 week, leaf tips were harvested every week, after 4 weeks harvesting of whole plant

Detection of WDV in the vector *Psammotettix alienus*



persistent transmission, non-propagative



Hougenhout et al. 2008, modified

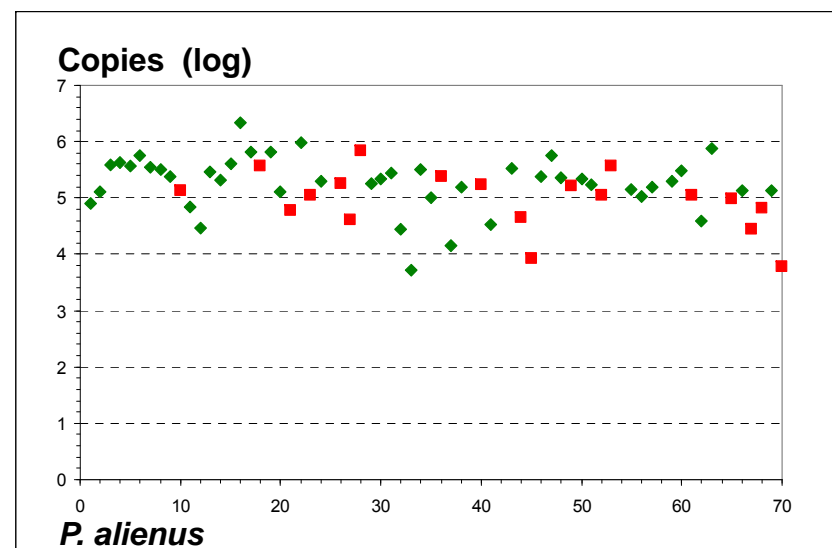
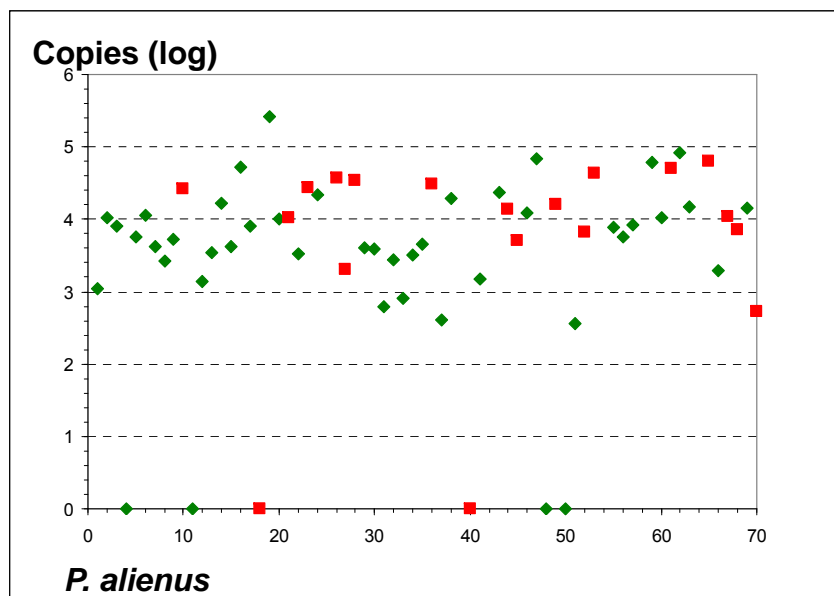


top: *P. alienus* stored in ethanol

bottom: *P. alienus* stored at -80°



Detection in *Psammotettix alienus*



n=64, winter barley ‚Rubina‘ inoculated with one viruliferous leafhopper for 7 days, afterwards analysis of leafhoppers by qPCR, plants were tested for infection after 4 weeks, **red** – WDV-positive plant, **green** – WDV-negative plant (tested with qPCR, viral load of plants not plotted).

Summary and outlook

-qPCR assays were developed which allow sensitive and specific detection and quantification of cereal geminiviruses

Application of the method:

-Monitoring the spatial and chronological development of viral infection of the plant

-Investigations of transmission behaviour of the vector

-Comparisons of virus titre of transgenic and non-transgenic wheat lines (mildew-resistant wheat developed at the University of Zürich)

A photograph of a grassy area with a ladybug on a blade of grass. The background is a close-up of green and brown grass blades growing from dark soil. A small red ladybug is visible on one of the green blades.

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