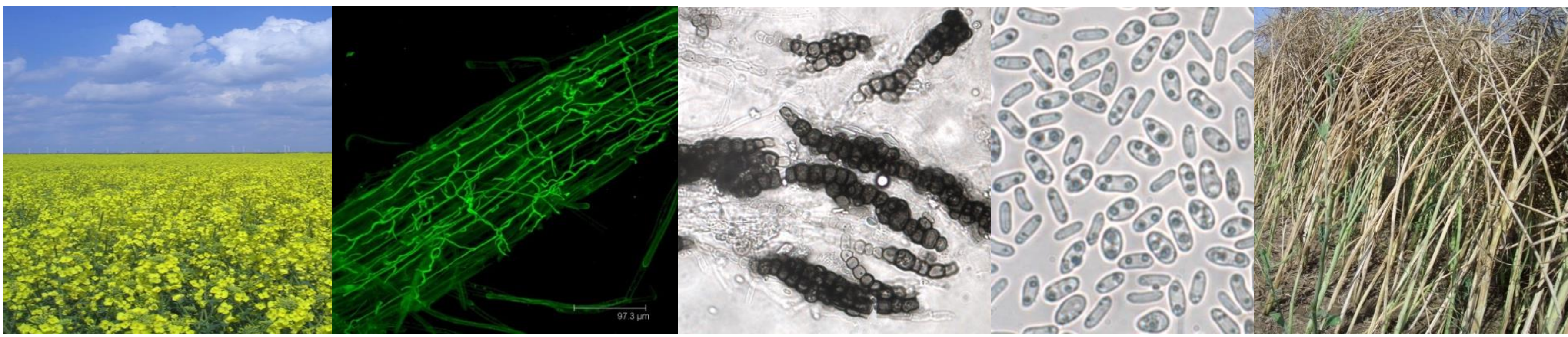
GCIRC Technical Meeting, Malmö, Sweden, May 8t – 11th 2017

Novel insights into the life cycle and epidemiology of Verticillium stem striping



Alice B. Eseola, Annette Pfordt, Xiaorong Zheng, Antonia Wilch, Birger Koopmann, Daniel Lopisso, Andreas von Tiedemann

Plant Pathology and Plant Protection Division, Department of Crop Sciences, Faculty of Agriculture,
Georg-August University of Göttingen, Germany

Novel insights into the life cycle and epidemiology of *Verticillium* stem striping

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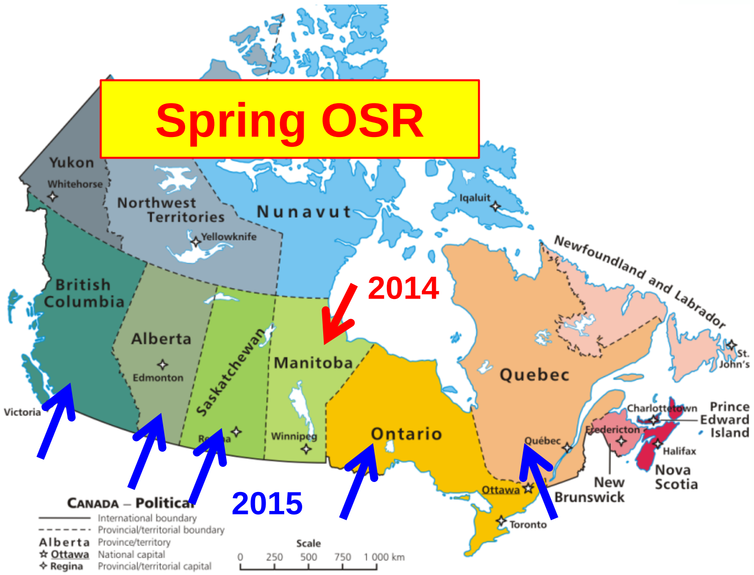
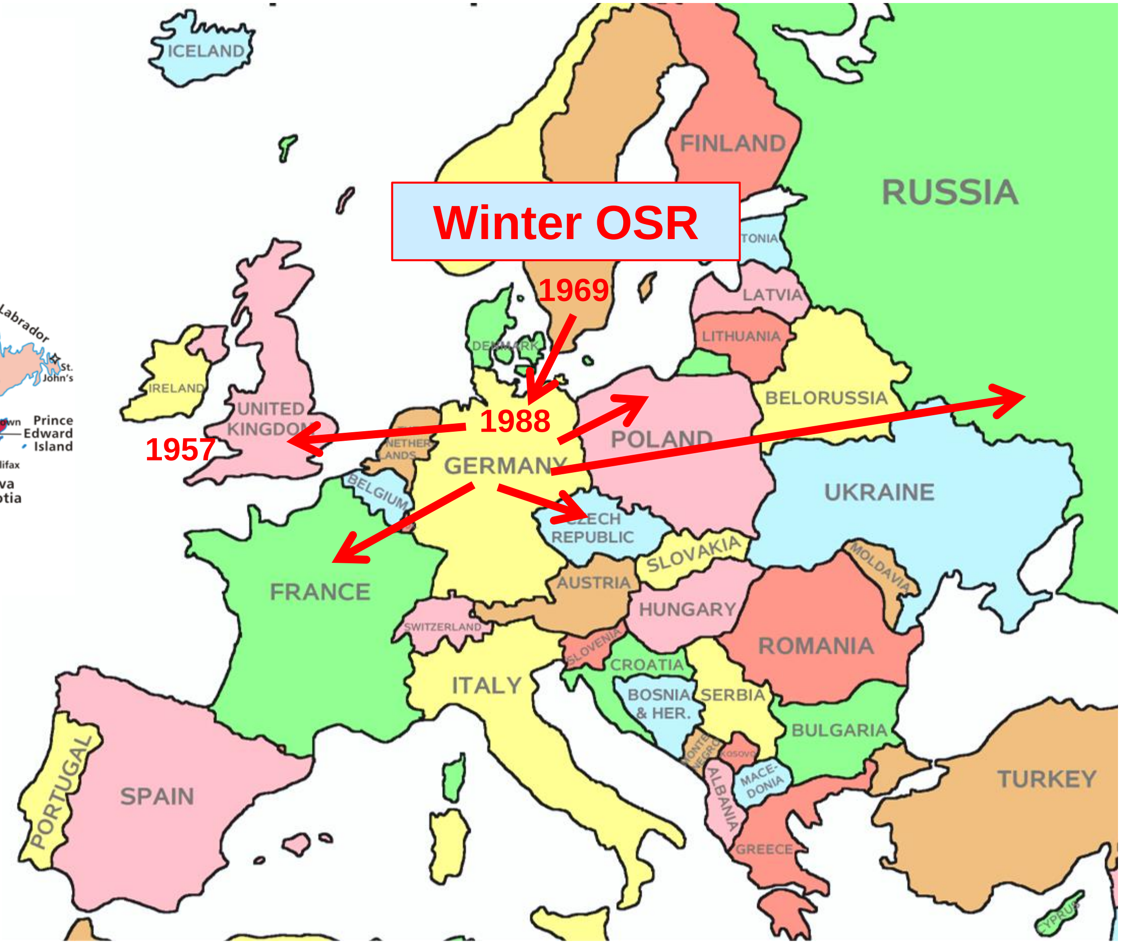
Verticillium longisporum (VL) is a soilborne vascular pathogen which causes stem striping in European and Canadian oilseed rape (OSR) and may induce significant yield losses. The epidemiological life cycle of VL is still not entirely understood, particularly referring to the time course of infection in the field and the transmission of the pathogen into the following rapeseed crop. We conducted field experiments with microsclerotia inoculated plots and assayed the colonization of VL during the season in spring and winter sown OSR cultivars contrasting in resistance to VL. Besides, soil temperature was varied in a miniplot soil heating experiment by +1.6 and +3.2°C above ambient. Enhancement of infection at increased soil temperature was paralleled with the accelerated colonization of spring type *vs.* winter type oilseed rape in the field, the earlier growing into the warm and the latter into the cold season. A climate chamber study revealed a minimum required soil temperature for stem invasion of 12°C in a susceptible and of 15°C in a moderately resistant interaction. The extended latency of VL infection and invasion of winter oilseed rape thus appears to be due to soil temperatures below 12-15°C in the early crop stages during autumn and winter. However, the further systemic spread of VL in the plant appears independent from environmental factors and mainly governed by the nutritional conditions in the xylem. In artificially inoculated winter OSR in the greenhouse, VL was transferred into the seeds to a higher rate in susceptible than in resistant cultivars. This was demonstrated on surface sterilized seeds using 3% sodium hypochlorite and Ds-RED labeled transgenic strains of VL. Similar results were obtained with spring OSR. These results obtained in artificial conditions suggest VL to be seed transmissible, however, the transmission of the disease from seeds in the field awaits further clarification. As a further pathway into the next season, we identified transmission by colonization of alternative hosts used as cover crops.

Global spread of *Verticillium longisporum*





First report on *V. longisporum* (supposedly), Brussels sprout, England, 1957:
Isaac, I. 1957 Verticillium wilt of Brussels sprout. *Ann. Appl. Biol*. 45, 276-283.

First report of *Verticillium* in oilseed rape, Sweden 1969:
Kroeker, G. 1970. Vissnesjuka på rabs och rybs i Skåne orsakad av Verticillium. In English: Verticillium on oilseed rape and turnip rape in Scania caused by Verticillium. *Svensk Frötidning*, 19, 10-13.

‘Verticillium stem striping’

There is no ‚verticillium wilt‘ in oilseed rape!

Microsclerotia

Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY DOI: 10.1111/mpp.12350

Pathogen Profile

Verticillium longisporum, the invisible threat to oilseed rape and other brassicaceous plant hosts

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SUMMARY

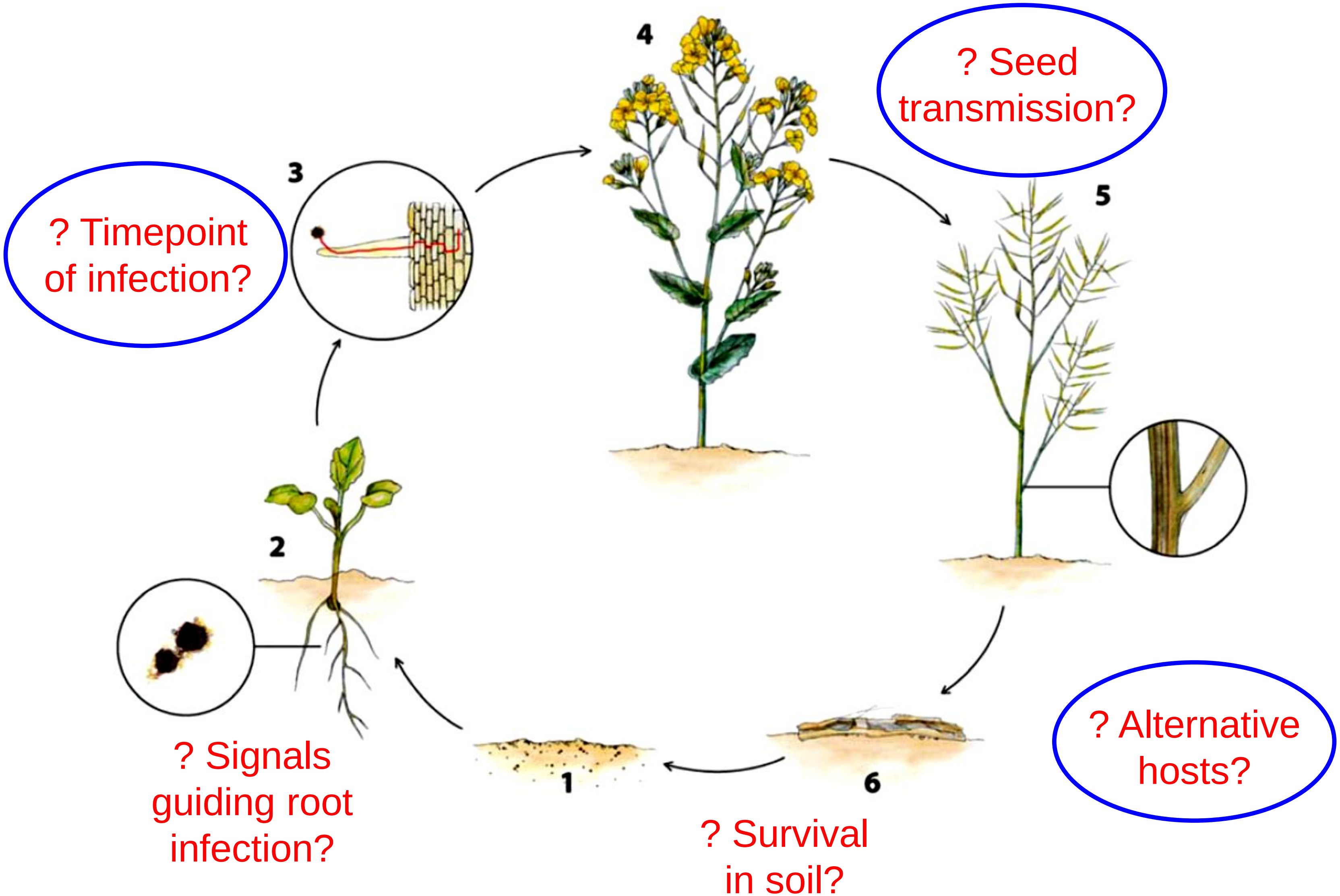
Introduction: The causal agents of Verticillium wilts are globally distributed pathogens that cause significant crop losses every year. Most Verticillium wilts are caused by *V. dahliae*,

we propose 'Verticillium stem striping' as the common name for *Verticillium* infections of oilseed rape.

Keywords: amphidiploid, *Arabidopsis*, *Brassica*, host range, pathogenicity, disease management, vascular wilt.

MPP, 17(7), 1004-16, 2016

V. longisporum – the knowledge gaps



Depotter *et al.*, *MPP*, 17(7), 1004-16, 2016

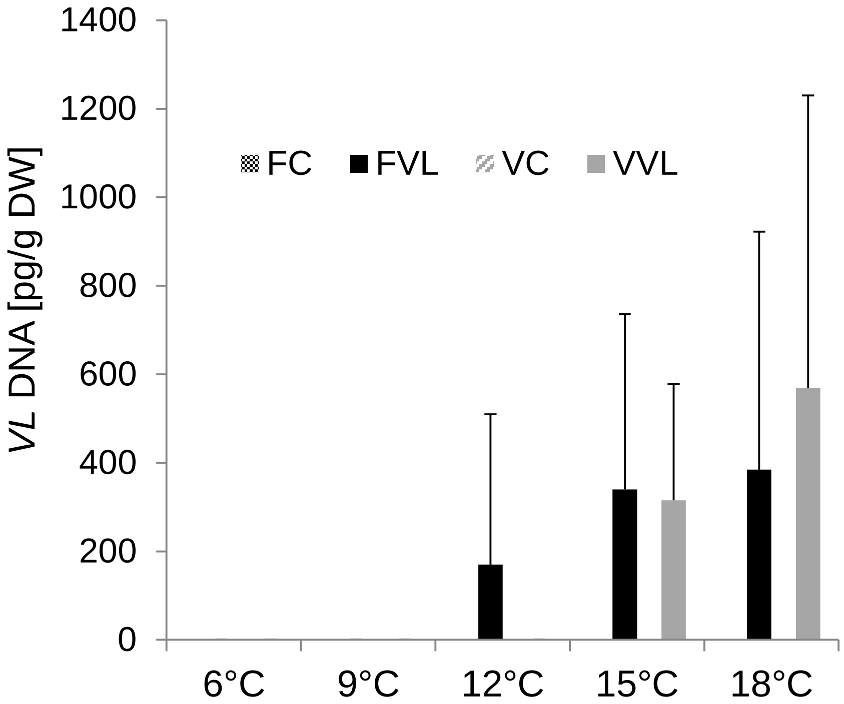
Progress of infection - winter oilseed rape

- 15 g Inoculum/m^2
- 5 plants/plot x 4 replicates
- qPCR, ß-tubulin primer

Sowing date:
27 August 2015

Effects of temperature on VL infection

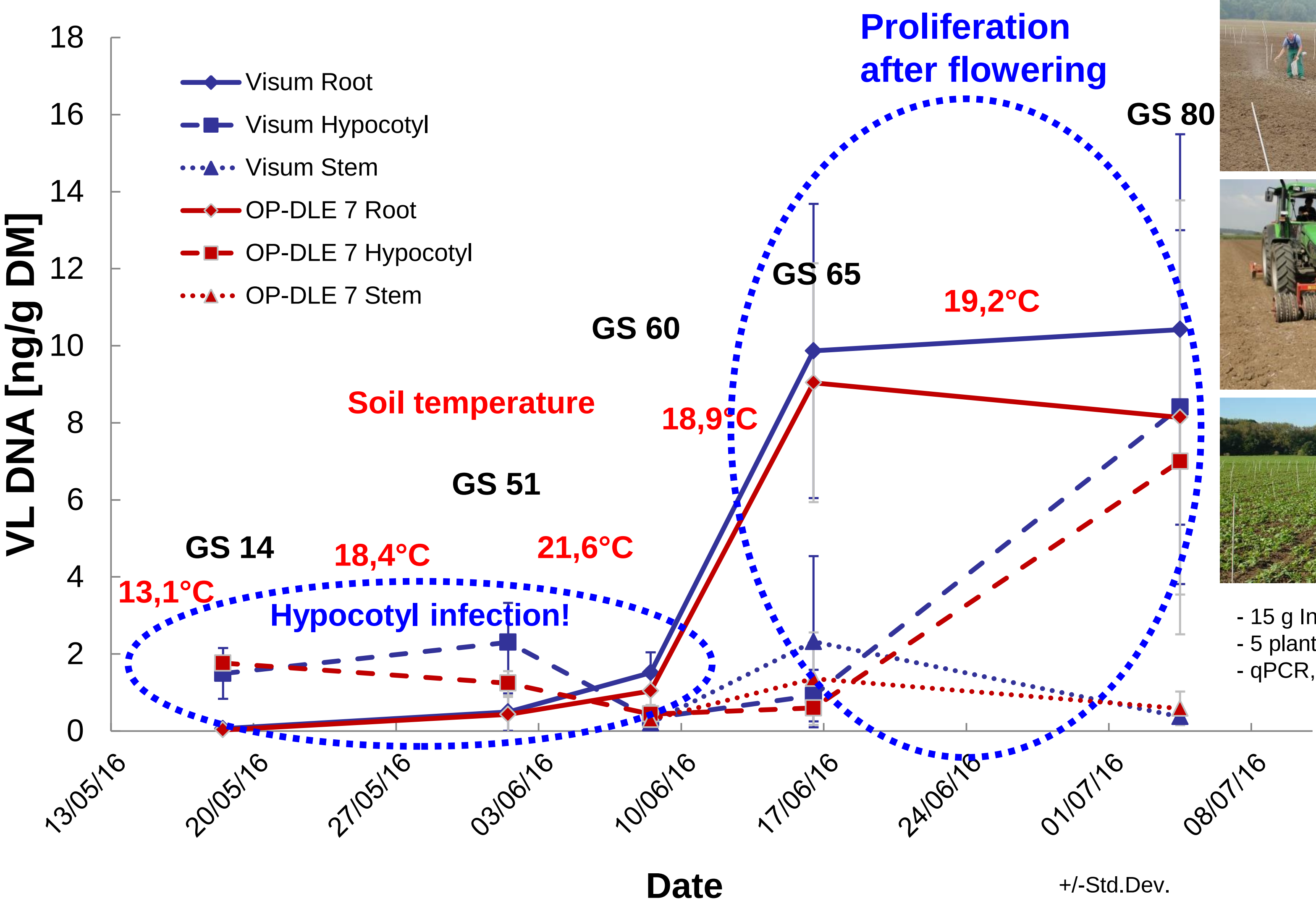
(climate chamber exp.; 45 dpi)



Msc thesis Annette Pfordt, 2016

Inoculation with 20 mg microslerotia per kg soil; F = Falcon (WOSR), V = Visum (SOSR)

Progress of infection – spring oilseed rape



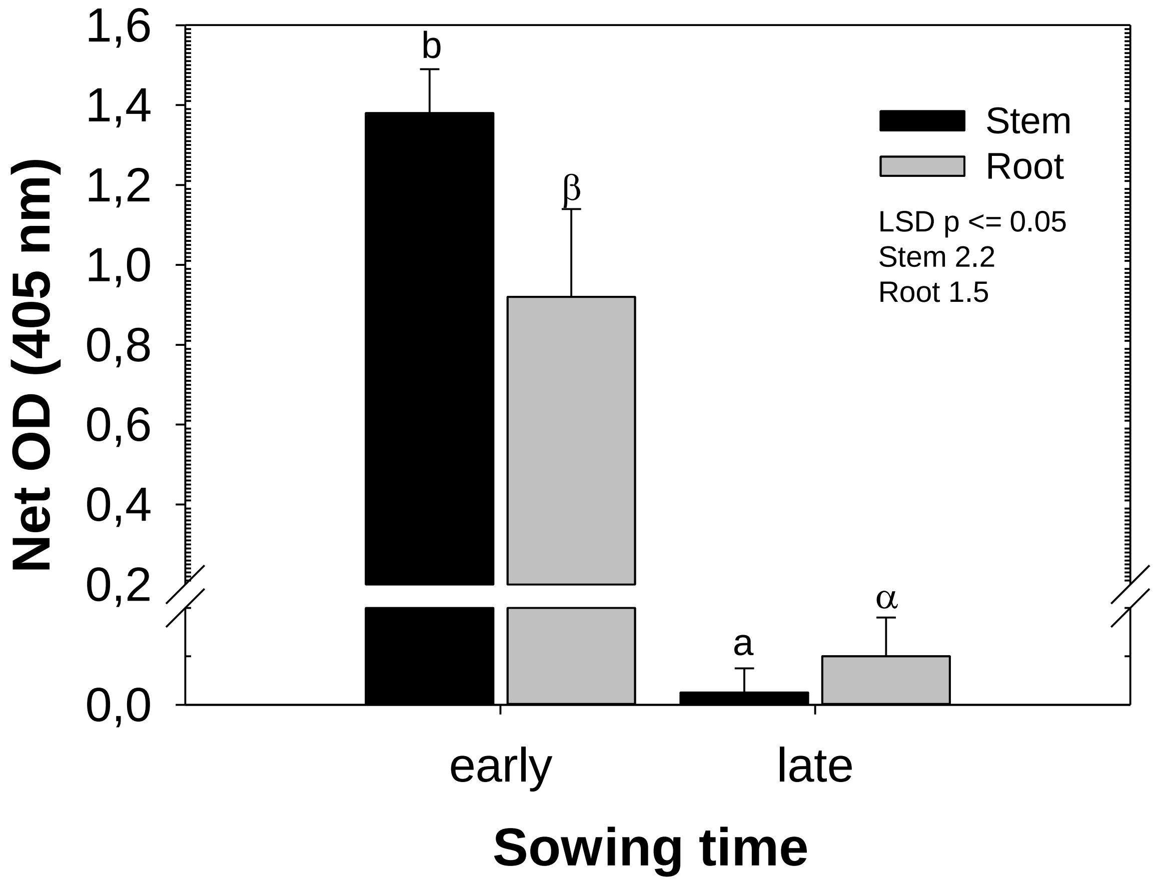
- 15 g Inoculum/m²
- 5 plants/plot x 4 replicates
- qPCR, ß-tubulin primer

Sowing date:
7 April 2016

Sowing time

Stem/root colonization with VL (ELISA)

Field trial 2006, GS 87, cv. ‚Oase', Weende, 15 g inoculum/m²

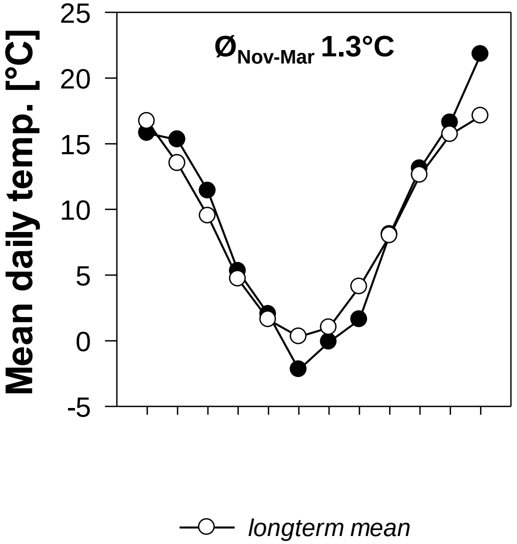


Early: 12. AUG 2005
Late 25. AUG 2005

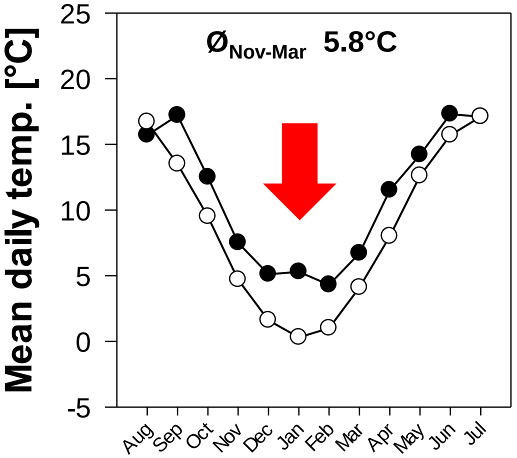
PhD thesis Harald Keunecke, 2009

Cabbage fly

Impact of winter temperature & cultivar



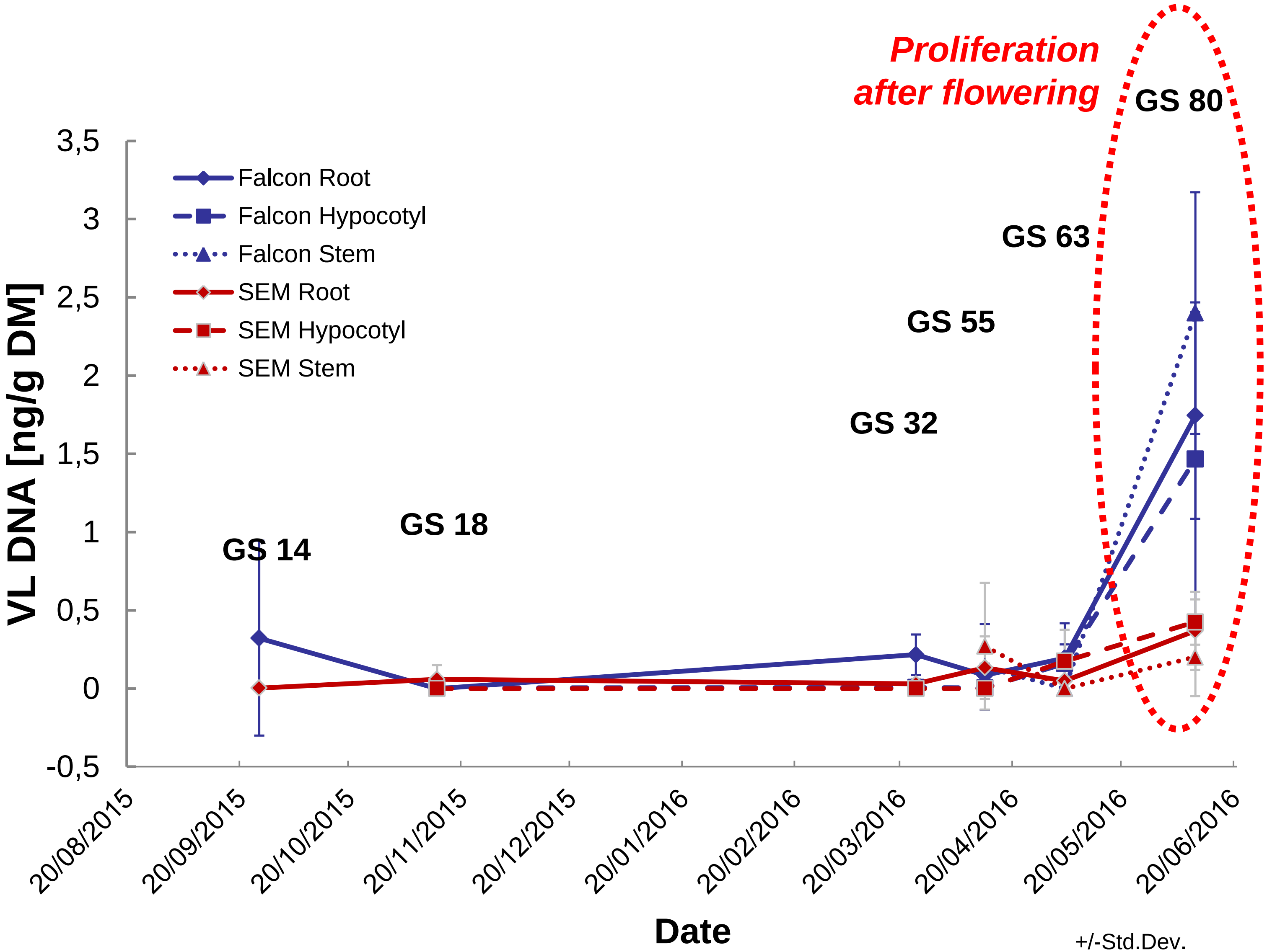
2005/06



2006/07

PhD thesis Harald Keunecke, 2009

Progress of infection - winter oilseed rape

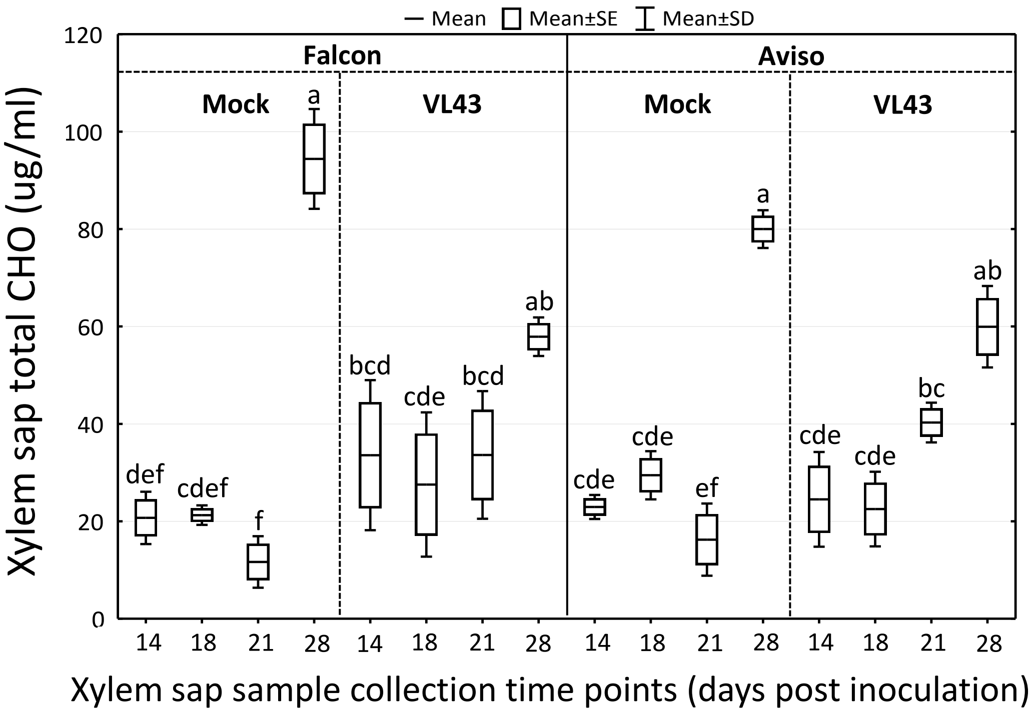


- 15 g Inoculum/m^2
- 5 plants/plot x 4 replicates
- qPCR, ß-tubulin primer

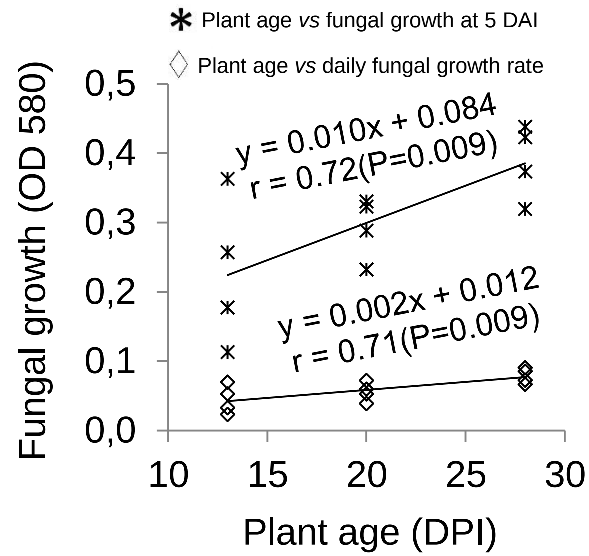
Sowing date:
27 August 2015

VL growth in xylem sap

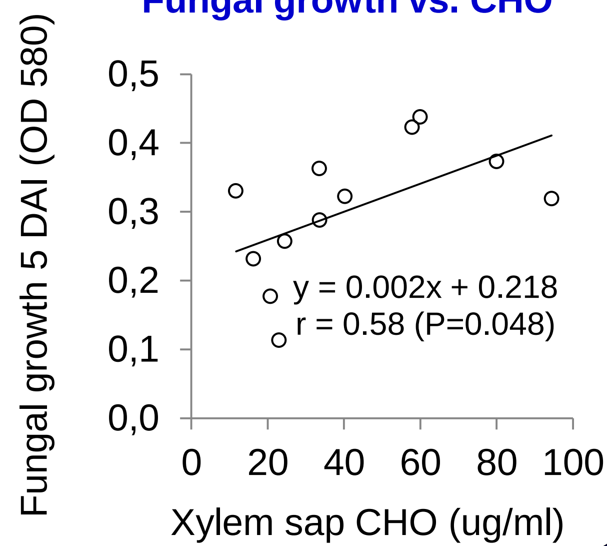
Carbohydrates in xylem *vs.* plant age

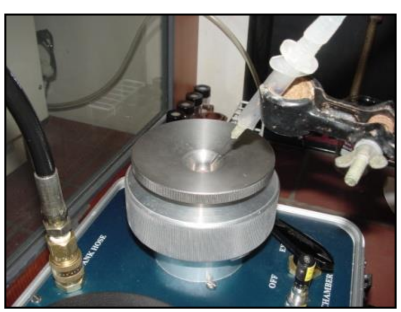


Fungal growth *vs.* plant age



Fungal growth vs. CHO





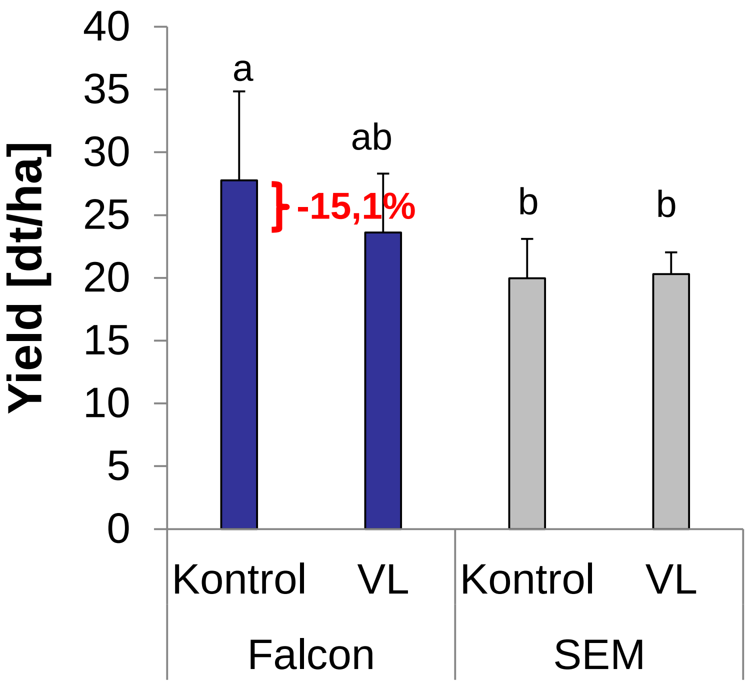
Growth of *Verticillium longisporum* in *Brassica napus* xylem sap is independent from cultivar resistance but promoted by plant ageing

Daniel T. Lopisso, Jessica Knüfer, Birger Koopmann, Andreas von Tiedemann

(submitted to *Phytopathology)*

Yield & quality – winter OSR

Field exp. Gö-Weende 2015/16

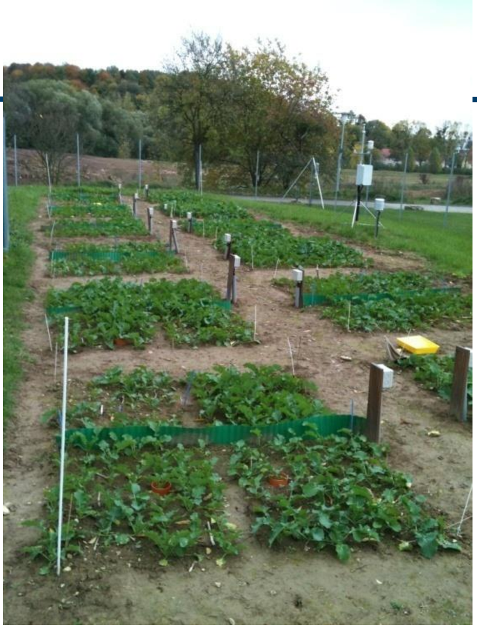


Yield & quality, spring OSR

Spring oilseed rape field exp. Weende 2016 destroyed by pollen beetle

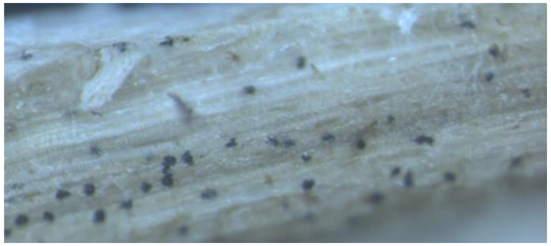
Role of cover crops in VL propagation

(greenhouse & miniplot field exps., 2016)



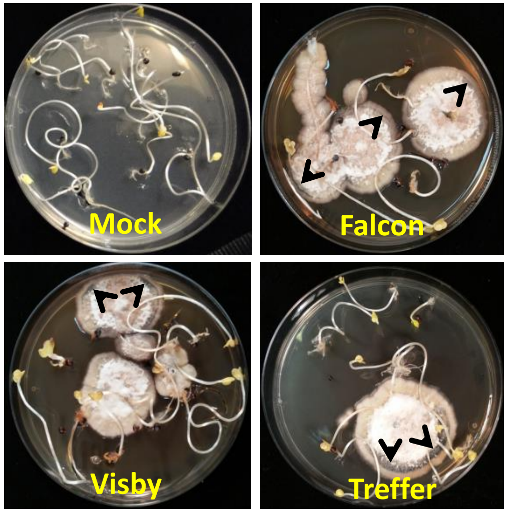
Cover crop	% plants with MS (greenhouse)	MS detected on field-grown plants
Mustard (*Sinapis arvense*)	**100**	na
Oil radish (*Raphanus sativus*)	**100**	**+**
Turnip rape (*Brassica rapa*)	**100**	na
Tansy phacelia (*Phacelia tanacetifolia)*	**72**	**+**
Winter rye (*Secale cereale*)	**0**	na
Italian clover (*Trifolium incarnatum*)	**0**	na

n. a. – not analysed in the field

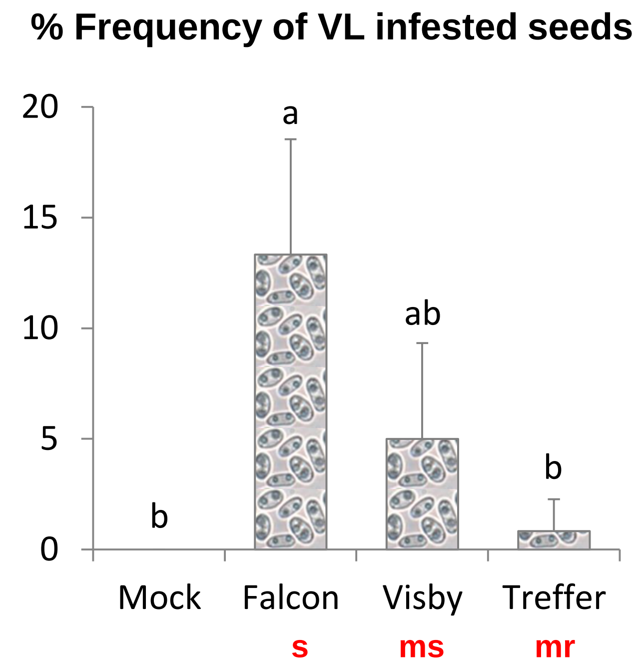


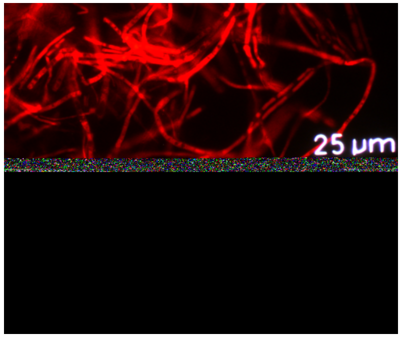
Bachelor thesis Sarah Bartsch, 2016

Seeds from greenhouse inoculated plants



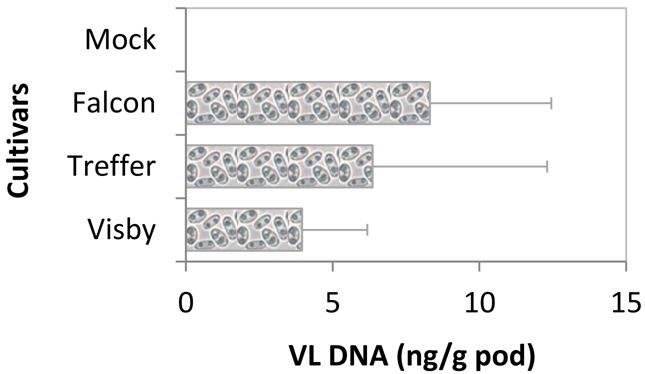
Re-isolation of VL from seeds harvested from inoculated plants.

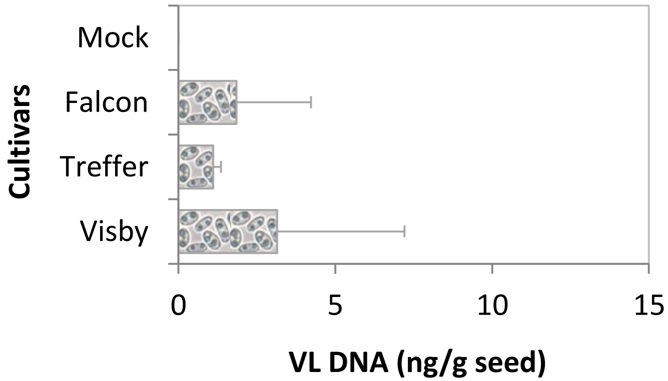




DsRed labelled strains of VL detected on seeds.

VL DNA in pods & seeds





Msc thesis Alice Bisola Eseola, 2016

Summary

- 'Verticillium stem striping' is a growing threat to global oilseed rape production.
- Extended latency in colonization of winter OSR, early spread into roots/hypocotyls in spring OSR.
- Early root/plant colonization apparently determined by soil temperature (threshold 10-12°C).
- Nutritional changes in the xylem sap may determine subsequent post-anthesis proliferation of VL into the shoot.
- Significant yield losses occur at/above 60% DI: 15-20% (WOSR).
- Phacelia, mustard, oil radish and turnip rape are alternative hosts of VL. → *Pathotype selection?*
- Seed transmission proven on greenhouse inoculated plants.
  Seed transmission in the field awaits confirmation.

The *Verticillium* group (2005-2017)

Sarah Dunker   Christina Eynck

Harald Keunecke

Nadine Riediger

Jessica Knüfer

Daniel Lopisso

Maggie Siebold

Xiaorong Zheng

PhD students

Antonia Wilch

PIs, Postdocs

Birger Koopmann

Avinash Kamble

Bachelor- und Master students

Eiko Tjaden, Fluture Novakazi, Sarah Bartsch, Alice B. Eseola

Lab technicians

Evelin Vorbeck

Jutta Schaper

Isabel Müller

Annette Pfordt

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Cooperation partners

Petr Karlovsky, Univ Göttingen
Heiko Becker & Christian Möllers, Univ Göttingen

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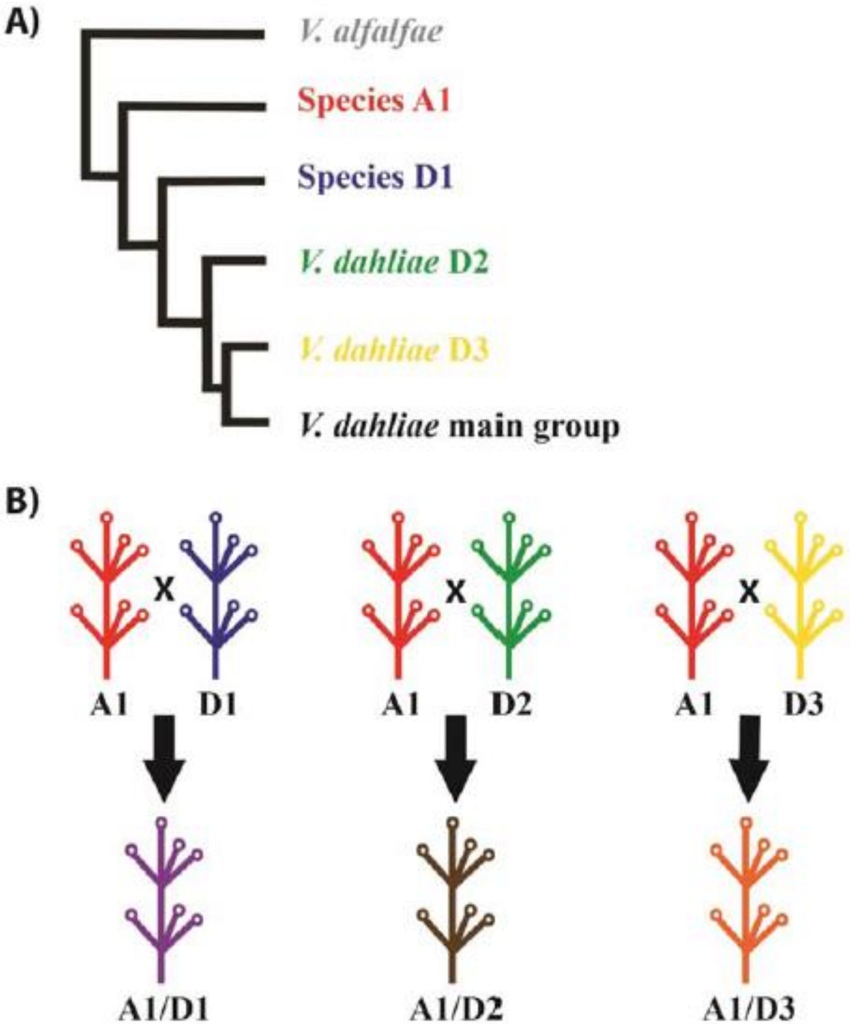
DFG – German Research Foundation

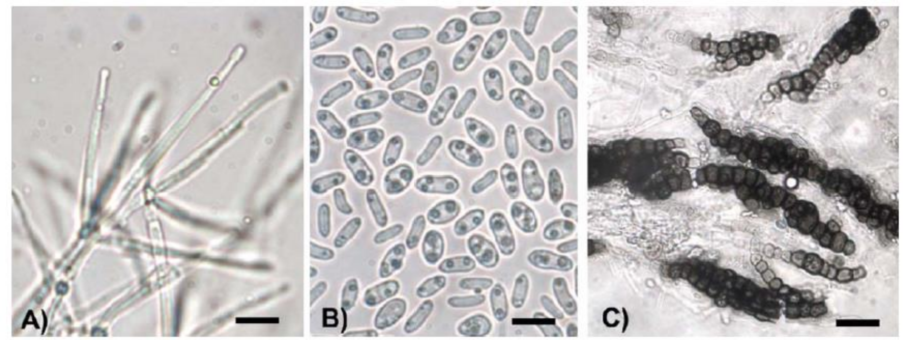
GFPi – German Association for the Promotion of Plant Innovation

BMEL/BLE Federal Ministry of Food and Agriculture

BMBF Federal Ministry of Education & Research

VL lineages = pathotypes?



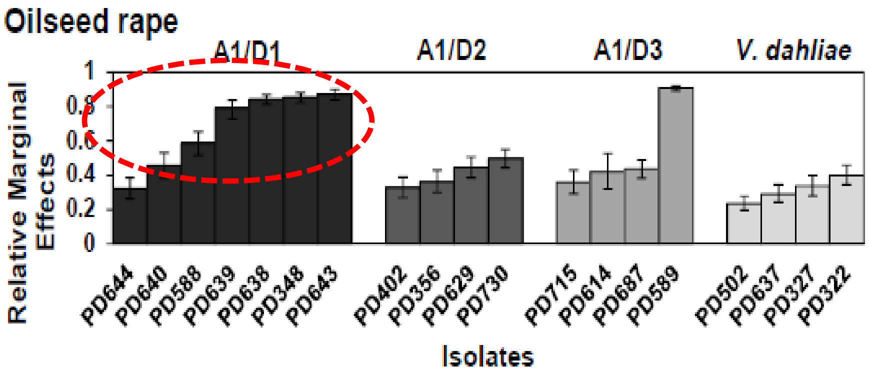


Line-age	Hosts	Countries
A1/D1	OSR, cauliflower, cabbage, birdrape, sugar beet	France, Germany, Sweden, Japan, USA (only cauliflower); Canada (OSR)
A1/D2	Horseradish; OSR	Illinois, USA, Sweden, Germany
A1/D3	OSR, cabbage, horseradish	Germany, Japan, France

Depotter et al., *MPP*, 2016

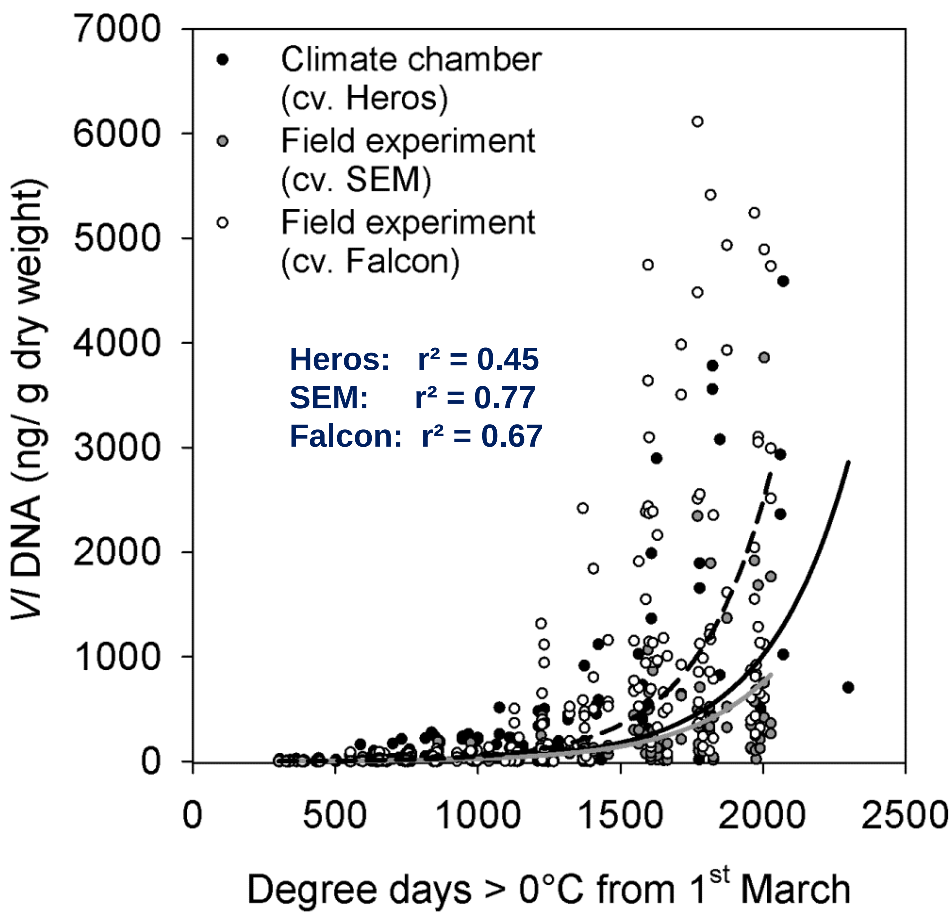
VL lineages - host range

Brassicaceae



Novakazi et al., *Phytopathology* 105: 662-673, 2015

Impact of soil temperature

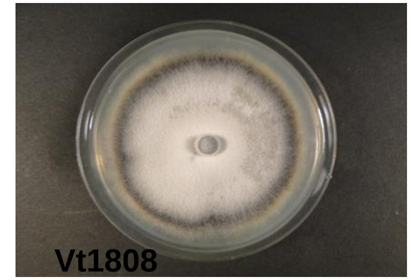


Göttingen Soil Heating Experiment

Siebold & Tiedemann (2013) *Global Change Biology,* 19: 1736-1747.

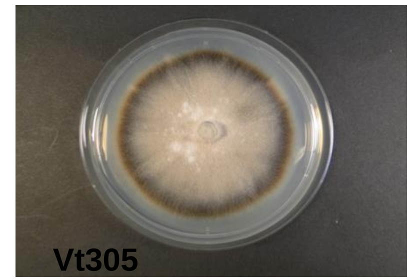
Biocontrol of *V. longisporum*

Verticillium tricorpus 1808



Source: Olive mill waste (OMW) compost/California, USA.
MoA: Unknown yet.

Verticillium isaaci (Vt305)



Source: suppressive soil in cauliflower field in Belgium.
MoA: competition for infection sites and ISR? Tryptophan-derived metabolites produced by the infected roots (root defence)? (Tyvaert *et al.* 2014)

Piriformospora indica

Order: Sebacinales
Source: Indian Thar desert
Mechanism: Resistance induction: (Lahrmann *et al.*, 2015)
Plant growth promotion: (Lahrmann *et al.,* 2012)

Rhizobium radiobacter F4

Source: Endofungal bacterium in *P. indica*
MoA: Jasmonate-based ISR (similar to Pi). (Glaeser *et al.,* 2015)

Master thesis project Dima Alnajar, 2016

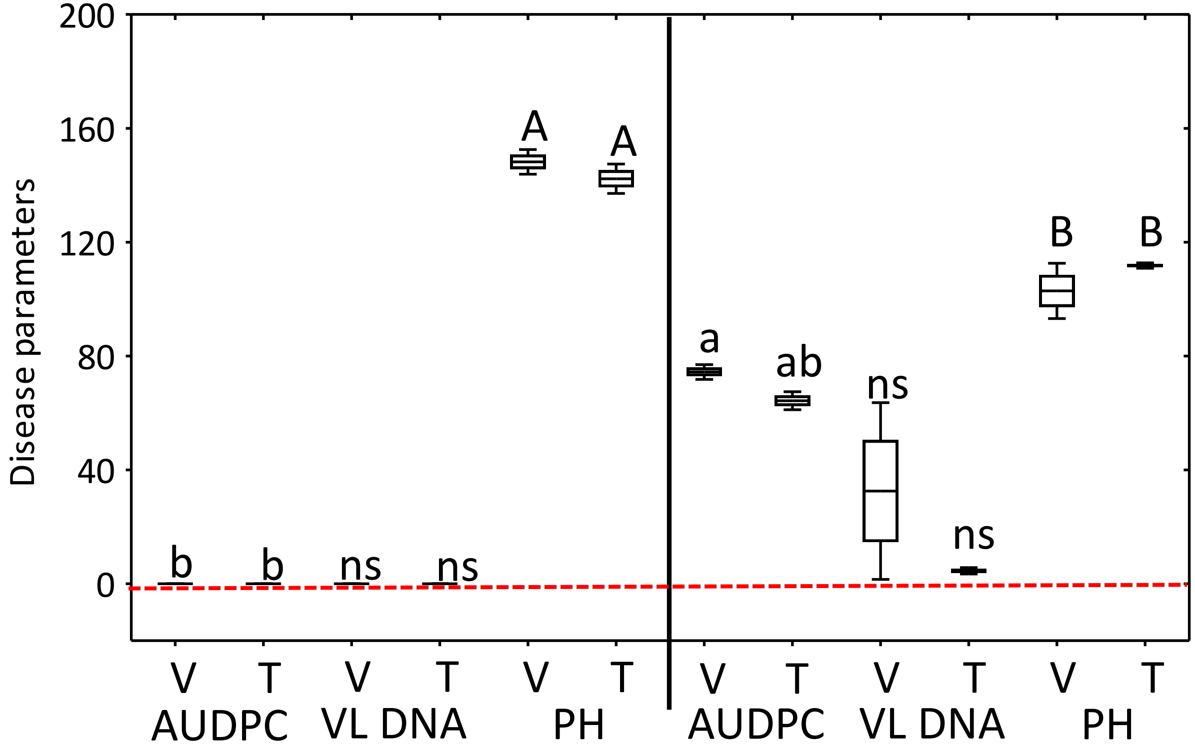
Isolates used in multiplex PCR. All isolates were obtained from the culture collection of the University of Göttingen
(Masterarbeit F. Novakazi, 2013)

Isolate	Pathotype	Origin	
		Crop	Country/Region
IPP 0109	**D1**	*Brassica napus*	Sweden
IPP 0127	**D1**	*Brassica napus*	Sweden
IPP 0128	**D2**	*Brassica napus*	Sweden
IPP 0129	**D1**	*Brassica napus*	Sweden
IPP 0450	**D1**	*Brassica napus*	Pöhl, Germany
IPP 0453	**D2**	*Brassica napus*	Rostock, Germany
IPP 0462	**D1**	*Brassica napus*	Hohenlieth, Germany
IPP 0467	**D1**	*Brassica napus*	France
IPP 0793	**D1**	*Brassica napus*	France
IPP 0794	**D1**	*Brassica napus*	Göttingen, Germany

In vitro and *planta* analysis of seeds from the field

Testing seeds from OSR naturally infected in the field.

Seeds were incubated on PDA for 28 days at 23°C in the dark. None of the analyzed seeds (n= 450/cultivar) were found to be contaminated or infected with VL.



V, T: cultivars ‚Visum',Treffer'
AUDPC: area-under-disease-progress-curve
PH: plant height
VL DNA: fungal DNA in plant hypocotyl

— Mean ☐ Mean±SE ⌶ Mean±SD

Msc thesis Alice Bisola Eseola, 2016