Laser-induced fluorescence spectroscopy in viticulture: An experimental study with Plasmopara viticola on potted vines under greenhouse conditions

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Motivation

- Pathogenic fungi (*Plasmopara viticola*, *Erysiphe necator*) severely threaten the annual yield of grapes in quantity and quality.
- Goal of current grapevine protection strategies: Reduction of fungicides by 50% until 2030 [1-3]:

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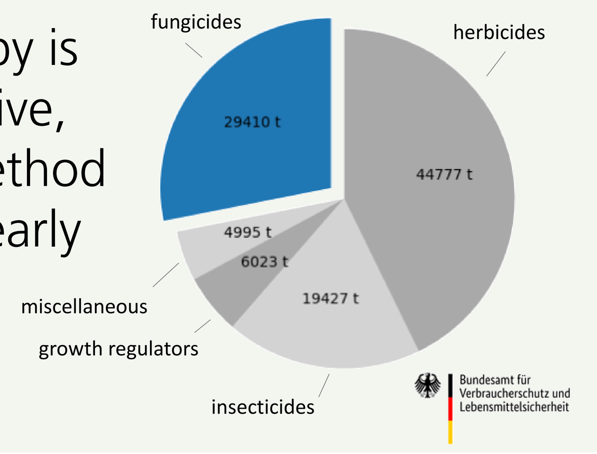
- A comprehensive, fast disease/pathogen assessment and fast monitoring tool is missing in viticulture.
- Optical spectroscopy is a rapid, cost-effective, non-destructive method with potential for early disease detection.



Aim of this work

RQ 1

- Is a reliable remote detection of specific pathogens in vineyards possible?
- Which system performance can be achieved with the vinoLAS® system?
- What are the key-figures compared with published (close-contact) systems [4-6]?

RQ 2

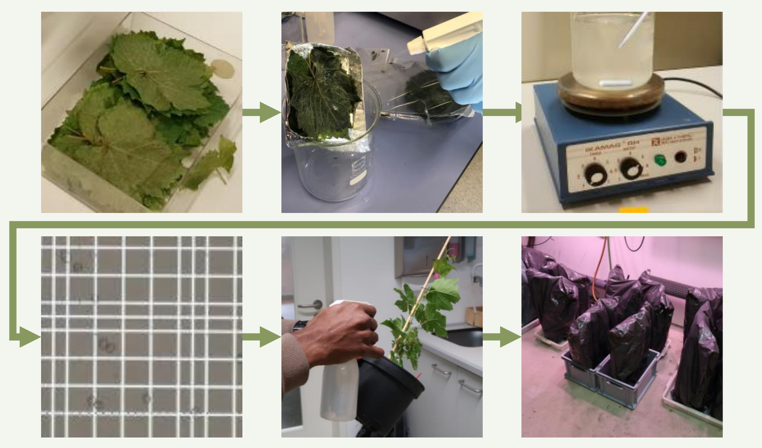
- To what extend does the pathogenesis over time affect the remote sensing and the fluorescence signals?
- After what time can first latent, asymptomatic infections be detected?

RQ 3

- Does the orientation of the leaf surface has an influence on the structure and strength of the fluorescence signal?
- What are the requirements for in-field operation?

Sample preparation

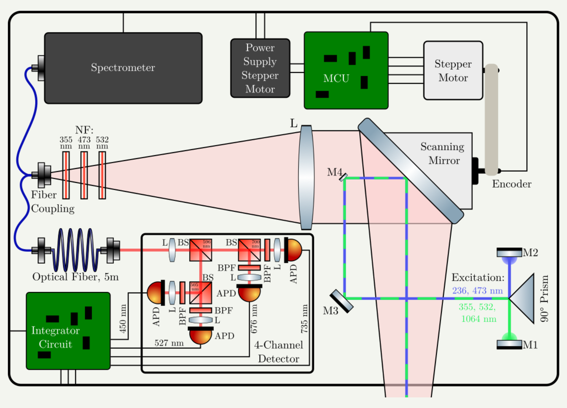
- 30 potted vines (Vitis vinifera L. cv. Riesling) grown in a greenhouse in standard potting medium:
  - inoculated group (*) (10 potted vines): treated with a sporangia suspension of P. viticola (concentration: $1 \cdot 10^5$ ml^{-1}),
  - non-inoculated group (*) (10 potted vines): treated with demineralized water,
  - validation group (10 potted vines): unknown state of inoculation of the individual plants for experimental operator.



(*) one individual potted vine was not watered (from both groups)

Experimental setup – vinoLAS®

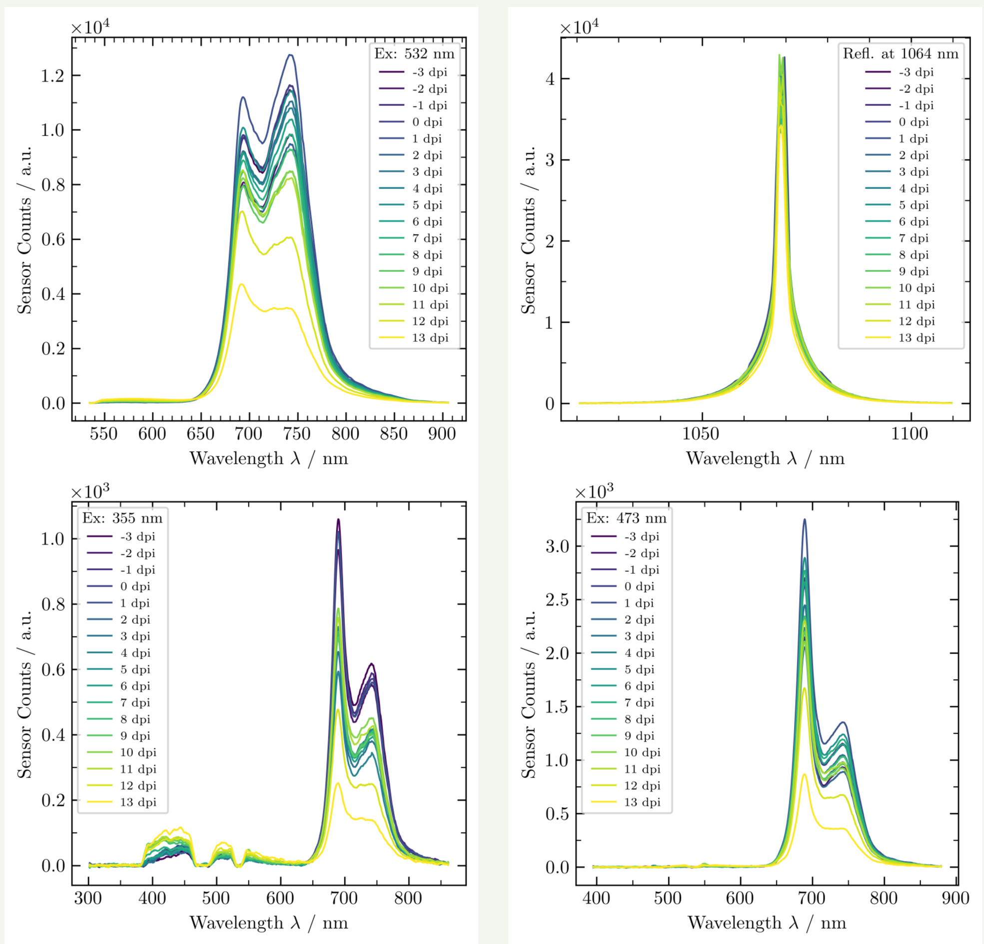
vinoLAS® is an innovative research model, especially tailored for fast non-invasive remote detection of pathogens in viticulture.



- Technology: Laser-induced fluorescence
- Passive q-switched nanosecond lasers:
  - excitation module 1: 236 nm, 473 nm
  - excitation module 2: 355 nm, 532 nm
  - reference wavelength: 1064 nm
  - rep. rate: 5 kHz, pulse energy: 0.34 µJ
- Detection distance: 1 m – 2 m (typ. 1.6 m)

Results – Temporal development of LIF

- Long-term measurements over 17 days
  Day of inoculation with *P. viticola*: 0 dpi

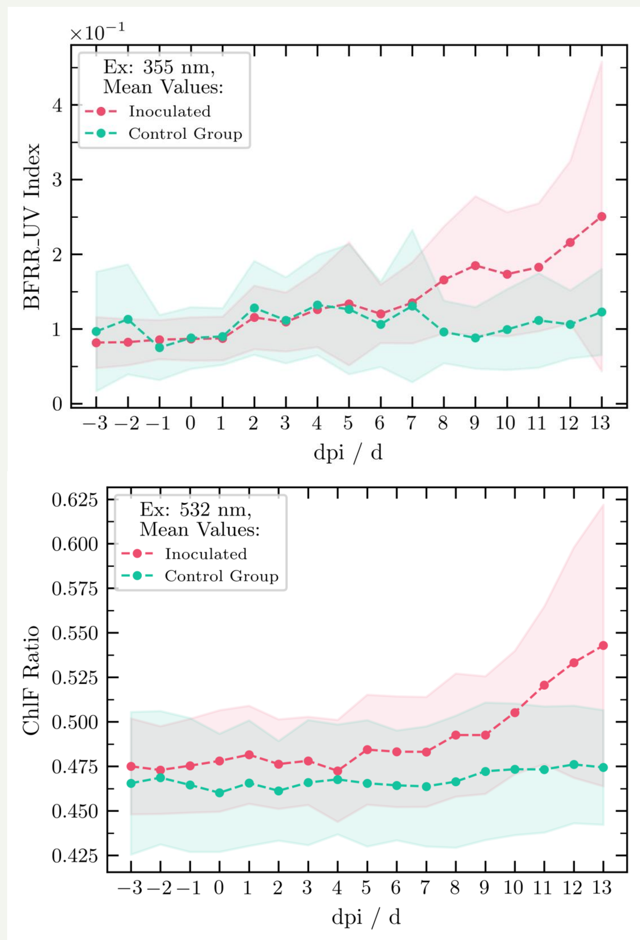


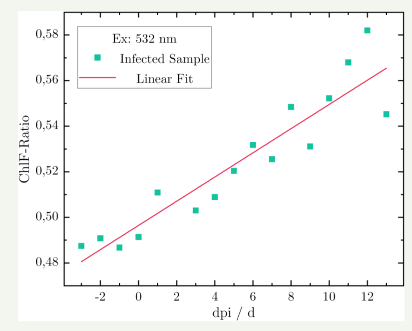
- Increase in blue-green fluorescence, measurable change of chlorophyll ratio

- Evaluation of integrated fluorescence bands by BFRR_UV index and ChlF-ratio:

$$\text{BFRR\_UV-Index} = \frac{F_{Blue}}{F_{Red2}}, \quad \text{ChlF-Ratio} = \frac{F_{Red1}}{F_{Red2}}$$

$$F_{Blue} = \int_{382nm}^{469nm} f(\lambda)d\lambda, \quad F_{Red1} = \int_{618nm}^{712nm} f(\lambda)d\lambda, \quad F_{Red2} = \int_{712nm}^{867nm} f(\lambda)d\lambda$$



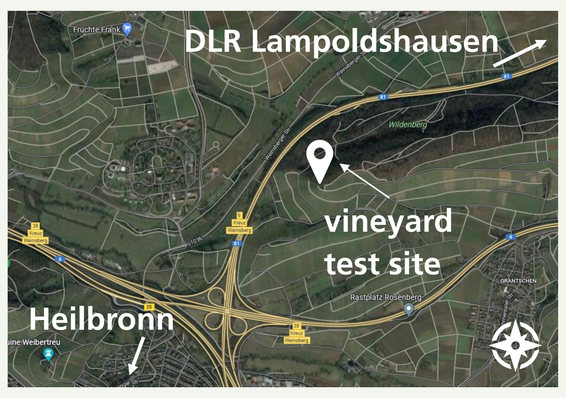


90% correct guesses

- Temporal change of BFRR_UV index and ChlF in case of infection after 7 dpi.

Results – In-field operation

- Test of vinoLAS® operational readiness in a trial vineyard



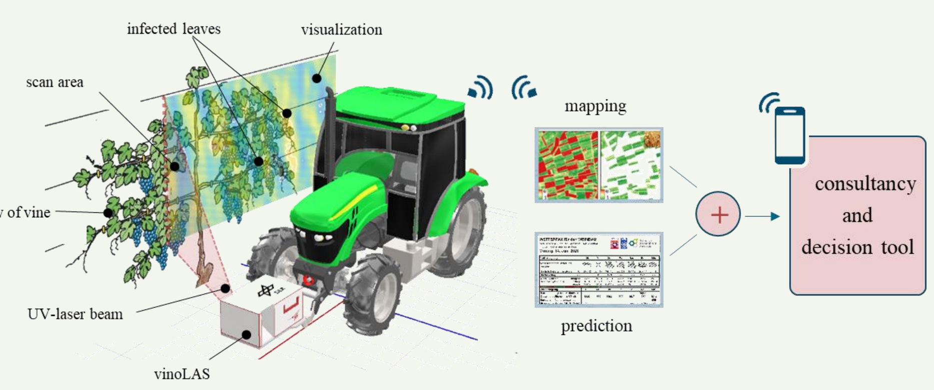
- Location: 49.164807° N, 9.307999° O
  24.8°C, 40% rH, sunny weather
- Requirements / readiness:
  - setup time: approx. 30 min
  - high robustness against vibrations
  - easy alignment of detection distance
  - protection against water and dust effects
  - strong influence by sun-induced autofluorescence

Results – Discussion

- Differentiation between healthy and an inoculated leaf tissue is possible (RQ 1).
- Pathogen symptoms are measurable by fluorescence detection after 7 dpi (RQ 2a).
- Hence, system can be used as a monitoring tool, but currently not for early pathogen detection (RQ 2b).
- LIF signal strength significantly varies between upper and lower leaf surface (factor 2.7) due to growth of sporangiophores on the lower side (RQ 3a).
- Detection capabilities have been elaborated in a field test. Influence of different grape varieties have to be considered in future studies (RQ 3b).
- By machine learning algorithms more information can be teased out of the vinoLAS system in future.

Outlook – Long term objective

- Automated disease monitoring in a vineyard using the vinoLAS® technology



- Long-term investigations in trial vineyards at different disease pressure levels
- Optimization of the vinoLAS setup (gated detection, multi-wavelength excitation)

References

[1] European Commission (2022). Proposal for a regulation of the European parliament and the council on the sustainable use of plant protection products and amending regulation (eu) 2021/2115 2022; **[2]** Eckpunkte des BMEL vom 29./30. November 2022; **[3]** Integrated plant protection according to guideline 2009/128/EG; **[4]** Ammoniaci, M., et al. (2021). State of the art of monitoring technologies and data processing for precision viticulture 11, 201. doi:10.3390/ agriculture11030201; **[5]** Bellow, S., et al. (2021). Optical detection of downy mildew in grapevine leaves: daily kinetics of autofluorescence upon infection. *Journal of Experimental Botany;* **[6]** Process, M., et al. (2023). An overview of chlorophyll fluorescence measurement process, meters and methods (IEEE)

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