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Research Note

Thermal sensitivity of grapevine leaves affected by *Plasmopara viticola* and water stress

M. Stoll[1)], H. R. Schultz[1)] and
B. Berkelmann-Loehnertz[2)]

[1)] Institut für Weinbau und Rebenzüchtung, Fachgebiet Weinbau, [2)] Institut für Biologie, Fachgebiet Phytomedizin, Forschungsanstalt Geisenheim, Geisenheim, Germany

K e y w o r d s : infrared thermography, stomatal conductance, biotic stress, pre-symptomatic diagnosis.

Introduction: There has been an increasing interest in using non-destructive thermal imagery to determine leaf temperature of plants. Leaf temperature has been proven as an indicator of plant responses to various stressors (Chaerle and van der Straeten 2000), in particular for plant water availability (Jones 1999). The theory is that a reduced stomatal aperture restricts transpiration and consequently heat dissipation, resulting in an increase in leaf temperature (Gates 1964).

The recognition of variations in leaf temperature led to the combined efforts to use infrared thermography and visible imagery to identify plant stress and non-destructively monitoring of plant's physiological status, particularly for plant water availability in grapevines (Jones *et al.* 2002, Stoll and Jones 2007) or in response to various pathogen attacks (Chaerle *et al.* 2004, Oerke 2006).

The research objective was to test whether infrared thermography could be used to distinguish between confined and infected versus non-infected areas upon attack by *Plasmopara viticola* in grapevine leaves under varying water status conditions.

Mateial and Methods: P l a n t m a t e r i a l a n d i n o c u l a t i o n : Measurements were conducted on grapevine leaves (*V. vinifera* L. 'Riesling') grown in a greenhouse. The different irrigation treatments were imposed prior to the start of the experiment. The treatments were: (a) control: irrigated and non-inoculated vines; (b) irrigated and inoculated vines; (c) non-irrigated and inoculated vines; (d): non-irrigated and non-inoculated vines. Watering was applied either every day or withheld during the entire experiment. The temperature in the greenhouse was set to 25 °C for daytime and 18 °C for night-time. Relative humidity and radiation were not controlled and no additional lighting was provided.

Three drops (each app. 30 µl) of a *Plasmopara viticola* sporangia suspension were added to the abaxial leaf surface at three intersections of main veins of each leaf covering an area of approximately 0.5 cm^2. Control plants were treated with 30 µl of tap water in place of the sporangia solution in the same configuration as for the inoculum on treated plants. Plants were covered over night with wet polyethylene bags. After 12 h the bags were removed and infrared images were taken every 24 h. On day eight, plants were kept in a darkened moist chamber over night at 100 % relative humidity to induce the sporulation of *Plasmopara viticola*.

T h e r m a l i m a g i n g : Thermal images were obtained using an uncooled focal plane array infrared camera (NEC TH7102 MX). The instrument operates in the waveband between 8-14 µm. The detector array has a geometric resolution of 1.58 m rad (320 x 240 pixels focal plane array and a 29° x 22° field of view lens with a minimum focus distance of 0.3 m). The thermal resolution is 0.06 °C and accuracy of absolute temperature measurement less than ± 2 °C. Images were analysed using the Pic-Win-IRIS software (ebs-GmbH, München).

L e a f g a s e x c h a n g e : Stomatal conductance of specific leaf positions, where inoculation had taken place, was measured with an open photosynthesis system (GFS 3000, Walz, Germany) using a leaf chamber of a cuvette area of 3 cm^2. Measurements were taken by selecting cuvette temperature and carbon dioxide set to ambient. CO_2 concentrations ranged from 440 to 455 $\mu mol \cdot mol^{-1}$. Airflow was fed through the chamber at 750 $ml \cdot min^{-1}$.

D a t a a n a l y s i s a n d s t a t i s t i c s : All experiments were conducted three times within one growing season. The effects of treatments were analysed by analysis of variance (Two Way ANOVA, with two levels of both factors) using R (R Foundation; University of Auckland, Nz).

Results and Discussion: R e l a t i o n s h i p b e t w e e n l e a f t e m p e r a t u r e , s t o m a t a l c o n d u c t a n c e a n d p a t h o g e n d e t e c t i o n : The interaction between *Plasmopara viticola* and the grapevine leaf was detected thermographically on 3 or 4 dpi (days past inoculation) before any visual symptoms occurred. In the greenhouse the leaf-air temperature difference (T_{leaf} - T_{air}) as a function of stomatal conductance was related for all treatments to the extent that higher leaf temperatures were associated with lower stomatal conductance. There were statistically significant differences ($P < 0.01$) in the slopes of the regressions when comparing inoculated and non-inoculated treatments. There was no statistically significant difference ($P = 0.132$) between the slopes of the regression when irrigated and non-irrigated treatments were compared. In irrigated plants the inoculation and development of the pathogen caused an increase in temperature at the position where inoculation had occurred (Figure, A). In contrast, under severe water stress the temperature at the inoculated areas further declined (Figure, B). This is in agreement with Allègre *et al.* (2007) who found that the mean temperature of totally colonized leaves declined during water stress. In addition, these authors showed that *Plasmopara viticola* induces an increase in stomatal aperture in the dark as opposed to the expected stomatal closure.

Correspondence to: Dr. M. Stoll, Forschungsanstalt Geisenheim, Institut für Weinbau und Rebenzüchtung, Fachgebiet Weinbau, von-Lade-Straße 1, 65366 Geisenheim, Germany. Fax: +49-6722-502140. E-mail: m.stoll@fa-gm.de

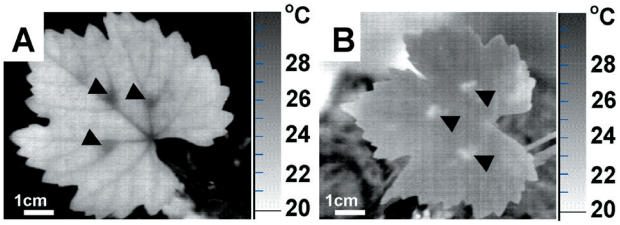

Figure: An example of a thermal image, presented in grey scale, on the fourth day after inoculation. Leaves (*Vitis vinifera* 'Riesling') inoculated with three drops of *Plasmopara viticola* zoosporangia on the lower leaf surface at three intersections of main veins of each leaf (black arrows). (**A**) Irrigated/inoculated leaf. (**B**) Non-irrigated/inoculated leaf.

Temporal sensitivity, temperature profile and maximum temperature difference: Histograms of the temperature profile can be used to demonstrate the spatial and temporal sensitivity. The temperature profile can be derived from the deviation of each individual pixel from the mean over a small distance measured along a straight line. Such analyses do not require the incorporation of environmental factors or reference temperatures. Temperature irregularities irrespective of the water status or the individual impact of changes in transpiration upon the fungal attack were found. The maximum temperature differences (MTD) were derived from 0.8 cm^2 circular areas of inoculated and non-inoculated leaf tissue. The MTD of inoculated leaves was higher than controls prior to the occurrence of any visual symptoms. For the irrigated/inoculated treatment, the MTD was highest on day six (0.89 °C) while for non-irrigated/inoculated vines the highest MTD was 0.31 °C on day 5.

If the area infected is small, any increase in size of the measuring area reduces the spatial information of the technique used. Even though the thermal resolution of the imager we used is stated as ± 0.06 °C, the accuracy is quoted as ±2 °C or an error of 2 %, whichever is larger. In our application, however, we are only concerned with relative variation in temperature so the thermal resolution is the more relevant. Indeed our approach has shown potential to reveal leaf temperature irregularities independent of environmental conditions and the absolute accuracy of the sensor.

Conclusion: The temperature profile shows spatial and temporal irregularities irrespective of the water status or of the individual impact of changes in transpiration upon the fungal attack. Since the technique interferes as little as possible with the leaves and the environment, infrared thermography can be seen as an interesting tool in the research of plant responses to stress. The information can be implemented as a first level of detection to identify non-specific pathogen development within the incubation period (pre-symptomatic), therefore determining the onset of plant stress.

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Allègre, M.; Daire, X.; Héloir, M. C.; Trouvelot, S.; Mercier, L.; Adrian, M.; Pugin, A.; 2007: Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves. New Phytol. **173**, 832-840.

Chaerle, L.; Hagenbeek, D.; de Bruyne, E.; Valcke, R.; van der Straeten, D.; 2004: Thermal and chlorophyll-fluorescence imaging distinguish plant-pathogen interactions at an early stage. Plant Cell Physiol. **45**, 887-896.

Chaerle, L.; van der Straeten, D.; 2000: Imaging techniques and the early detection of plant stress. Trends Plant Sci. **5**, 495-501.

Gates, D. M.; 1964: Transpiration and leaf temperature. Ann. Rev. Plant Physiol. **19**, 211-238.

Jones, H. G.; 1999: Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant Cell Environ. **22**, 1043- 1055.

Jones, H. G., Stoll, M.; Santos, T.; Sousa, C. D.; Chaves, M. M.; Grant, O. M.; 2002: Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. J. Exp. Bot. **53**, 2249-2260.

Oerke, E. C.; Steiner, U.; Dehne, H. W.; Lindenthal, M.; 2006: Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. J. Exp. Bot. **57**, 2121-2132.

Stoll, M.; Jones, H. G.; 2007: Thermal imaging as a viable tool for monitoring plant stress. J. Int. Sci. Vigne Vin. **41**, 77-84.

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