THERMAL SENSING OF ROSES AFFECTED BY DOWNY MILDEW

S. Gómez, E.-C. Oerke, H.-W. Dehne and U. Steiner

Institute for Crop Science and Resource Conservation (INRES) - Phytomedicine
University of Bonn
Bonn, Germany

ABSTRACT

Downy mildew caused by the oomycete *Peronospora sparsa* affects roses and is a serious problem in nurseries and cut roses in commercial greenhouses, especially in those without heating systems. The disease, which affects the quality and the yield of roses, develops fast under suitable environmental conditions. Currently it is controlled mainly by the application of foliar fungicides and removal of symptomatic plant material due to the limited availability of resistant cultivars of high commercial value. Though downy mildew is a destructive disease and its control increases production costs, few alternatives have been explored to detect the disease at early stages. In greenhouses epidemics begin usually in localized areas of the crop. Therefore, non-invasive systems for early detection of the disease may become an alternative in a future framework of more sustainable crop management. Hence, this study evaluated the visualization of rose downy mildew in adult plants by infrared thermography for presymptomatic detection of the disease. Roses of the cultivar Elle® susceptible to *P. sparsa* were inoculated, incubated and the presence and development of the disease was followed visually and thermographically. Mock inoculated plants were kept under the same conditions. Thermal assessments were conducted using a VARIOSCAN 3201 ST camera (Jenoptic Laser, Jena, Germany) with 0.03 K thermal resolution. Initial symptoms were detected visually five days post inoculation (dpi), while presence of the disease was visualized by thermal imaging one or two days before. Results showed that leaf temperature of plants was affected after the inoculation and its dynamics changed over time depending on the phase of the disease. Infection of *P. sparsa* in early stages of pathogenesis was detected as an increase of leaf temperature compared to healthy tissue, which indicated a decrease in leaf transpiration. The technique allowed the recognition of infected leaflets in the leaf, affected leaves in the plant and to discriminate between healthy and diseased plants. In contrast to other host-downy mildew interactions the response of roses to leaf colonization by *P. sparsa* was clearly associated with an increased leaf temperature throughout the pathogen development. The use of thermography for the detection of primary foci of diseased plants at the commercial level seems to be suitable because of this unambiguous host plant response. The technology may be applied in automated monitoring systems from above the crop canopy or from the side of plant rows, even on different scales. IR thermography proved to have a high potential as a non-invasive method for the detection of the disease and has become a promising tool to be used in risk assessment programs of rose crops in commercial production systems.

Keywords: non-invasive detection, early recognition, *Peronospora sparsa,* thermography

INTRODUCTION

Downy mildew caused by the oomycete *Peronospora sparsa* was first reported in England in 1862. The disease is a severe problem and may cause high losses in commercial greenhouses (Gullino and Garibaldi, 1996). Rose downy mildew is a destructive disease, especially in commercial rose crops in the tropics. Suárez (1999) reported losses due to the disease coming close to US$ 2,910 per hectare at the Bogota Plateau, Colombia.

The disease may be found where roses are cultivated (Xu and Pettitt, 2003). Leaves, stems, peduncles, calices and sepals can be infected, showing typically purple to brown lesions (Francis, 1981; Aegerter et al., 2002; Xu and Pettitt, 2003). The most prominent symptoms are those associated with leaves and stems (Alfieri, 1968) and the drop of severely infected leaves is common (Horst, 1983). Downy mildew development is favored by 90-100% relative humidity (RH) and relatively low temperatures (Wheeler, 1981). The pathogen requires moisture and it is difficult to control with fungicides (Karlik and Tjosvold, 2003).

Downy mildews are difficult to handle because their growth depends on living plant tissue. The lack of visible symptoms during early stages of infection makes difficult the visual detection of the disease at the field (Clark and Spencer-Phillips, 2004). Most rose growers regularly and systematically monitor and inspect visually plants or particular areas of the crop to detect the disease. Nevertheless, the control of the disease based on this detection method is not effective. Non-invasive sensor techniques like infrared thermography have been reported for monitoring of various plant-pathogen interactions. This technology, based on the principle that transpiration of water through stomata cools leaves and hence stomatal closure results in localized temperature increases is a promising alternative to study biotic factors causing stress in plants (Grant et al., 2006). As plant pathogens can influence stomatal aperture (Jones, 2004, Chaerle et al., 2004), the application of infrared thermography has been demonstrated for the early detection of plant responses in various plant pathogen interactions (Chaerle et al., 2004; Oerke and Steiner, 2010; Oerke et al., 2011; Wang et al., 2012).

Downy mildew of cucumber caused by *Pseudoperonospora cubensis* and grapevine downy mildew caused and *Plasmopara viticola* have been successfully detected by infra-red thermography before the appearance of disease symptoms (Lindenthal et al., 2005; Oerke et al., 2006; Stoll et al., 2008). Due to the localized beginning of rose downy mildew epidemics in greenhouses, non-invasive systems for early detection of the disease may become an alternative in a future framework of more sustainable crop management. The aim of this study was to assess the development of downy mildew infection in rose plants by thermography as a previous step to its use under commercial conditions.

MATERIALS AND METHODS

Plant material and pathogen inoculation. Rose plants cv. Elle® grown in glasshouse under 16 h photoperiod and average temperatures of 23°C/18°C (day/night) where used to evaluate the development of rose downy mildew by thermography. For the inoculation an isolate of *P. sparsa* from Colombia kept under controlled condition in the laboratory was used. The plants were inoculated when young shoots were developed spraying a suspension of 5.0×10^4 sporangia of *P. sparsa* per ml. As control, plants sprayed with distilled sterile water were used. After treatment, plants were kept under darkness for 48 hours at 10°C and 100% RH in a growth chamber to ensure pathogen infection. Then, the plants were transferred to 22°C/18°C day/night temperature, 16 hours of photoperiod and 60 ± 10% RH conditions for evaluation.

Disease assessment. Visual assessments started three days after inoculation (dai) and were made daily in order to establish the first appearance of disease symptoms. During the evaluation, samples of different symptoms were collected to induce sporulation to confirm the presence of *P. sparsa* under the stereoscope. A disease index (DI) was calculated as follows: $DI = [(0\times n) + (1\times n) + (2\times n) + (3\times n) + (4\times n) + (5\times n)] / \sum n$, where numbers show the categories presented in Table 1 and n represents the number of leaves observed under each category per plant. RBG images were recorded to illustrate the symptoms and their progress over time.

Table 1. Description of symptoms of downy mildew caused by *Peronospora sparsa* on rose plants cv. Elle® grouped in grades.

Grade	Description of symptoms on leaves
0	No symptoms
1	Dull leaflets, soft chlorotic spots and slightly disturbed leaves
2	Disturbed leaves, strong chlorotic spots and/or brown purple color angular shape
3	Highly disturbed leaves, strong and expanded brown spots, shrivelled leaves and desiccated areas or leaf borders

4	Strong brown spots, important desiccated leaf area (>60%), fall of leaflets
5	Strong spots, leaf completely desiccated, detached or absent

Thermographic evaluation. Starting three days after inoculation thermograms were recorded daily at a distance of 100 cm from the plants under study. Thermal images were obtained using an infrared Stirling-cooled scanning camera VARIOSCAN 3201 ST (Jenoptic Laser, Jena, Germany) with a spectral sensitivity from 8 to 12 µm and 1.5 m radians of geometric resolution (240 × 360 pixels focal plane array and a 30° × 20° field of view lens with a minimum focus distance of approximately 20 cm). Thermal resolution was 0.03 K, and accuracy of absolute temperature measurement was <±2 K. The software IRBIS® Plus version 2.2 (Infratec, Dresden, Germany) was used to analyze the digital thermograms. The average temperature of inoculated and non-inoculated leaflets for each thermogram recorded over the period of evaluation was calculated. The maximum temperature difference (MTD) of the leaves, which is provided by the IRBIS® program, was analyzed (Lindenthal et al., 2005; Oerke et al., 2006; Oerke et al., 2011; Wang et al., 2012). Data were analyzed statistically using the Superior Performing Software System SPSS 21.0 (SPSS Inc., Chicago, IL, USA) to conduct independent-sample t-tests analyses for means comparison and standard analysis of variance at a significance level of 95%.

RESULTS AND DISCUSION

Dull leaflets, chlorotic spots and slight leaf distortion were the initial changes visually detected on infected leaves 5 dai. Then, first symptoms evolved to typical disease symptoms such as brown-chlorotic spots, shrivelled leaves with extended brown-purple lesions and leaf drop. Presence of purple spots on sepals and brown spots with white waxy center appeared on the upper stems close to the floral peduncle. No symptoms were observed in non-inoculated plants. The severity of leaf symptoms increased over time and leaf spots and lamina distortion were followed by desiccated tissue and further drop of leaves 9 dai (Fig. 1).

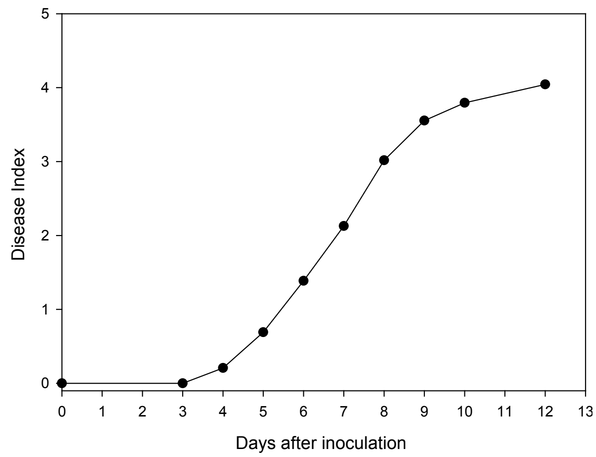

Figure 1. Progress of downy mildew in rose cv. Elle® assessed as the disease index following the inoculation of *Peronospora sparsa*.

Changes in leaf temperature of inoculated plants were detected as early as 3 dai (Fig. 2). For inoculated plants an increase of leaf temperature could be detected by IR thermography before visual disease symptoms were detected (Fig. 3). The average, maximum and minimum temperatures of infected plants were statistically higher than those of non-inoculated plants by 1.2 K 7 dai. Leaves of non-inoculated plants had similar temperatures throughout the experiment. Thermography allowed the discrimination between healthy and diseased plants (Fig. 3). In contrast to other plant-downy mildew interactions the response of roses to leaf colonization by *P. sparsa* was clearly associated with an increased leaf temperature throughout the disease development as presented in Fig. 2 and Fig. 3.

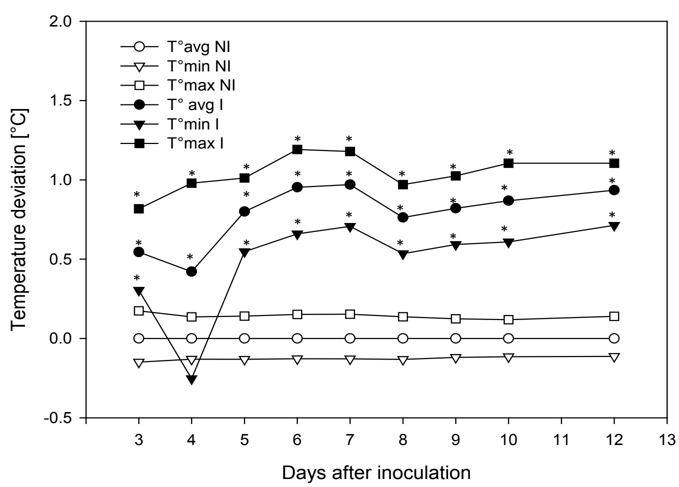

Figure 2. Effect of *Peronospora sparsa* infection on leaf temperature of rose leaves cv. Elle®. Average, maximum and minimum temperature of inoculated (I) and non-inoculated (NI) leaves represent differences in temperature related to

average temperature of non-inoculated leaves. Asterisks show values significantly different from non-inoculated leaves (n = 12; t-test, $P < 0.05$).

For other downy mildews, Lindenthal et al. (2005) recorded thermal changes of the infected tissue of cucumber one day before the appearance of disease symptoms of *Pseudoperonospora cubensis*. Stoll et al. (2008) also detected *Plasmopara viticola* infection before the appearance of visual symptoms in grapevine. The infection of rose leaves by *P. sparsa* in early stages of pathogenesis detected in the thermograms as an increase of leaf temperature compared to healthy tissue, may indicate possibly a decrease of leaf transpiration. Perturbation of transpiration may be used as signal for the development of plant diseases affecting stomatal aperture and cuticle damage (Oerke and Steiner, 2010). It has been described for downy mildew of cucumber and apple scab that a decrease in temperature of infected leaf tissue is caused by evaporation of leaf water as a consequence of damage of plant cuticle (Lindenthal et al., 2005; Oerke et al., 2006). In contrast, in the current study an increase of infected leaves temperature was observed soon after *P. sparsa* infection and during most of the development of the disease. Pathogens can influence stomatal aperture by interposing water transport or by releasing specific compounds that induce plant responses (Jones, 2004, Chaerle et al., 2004). Nevertheless, additional studies are required to identify factors involved in thermal responses of rose plants to *P. sparsa* infection.

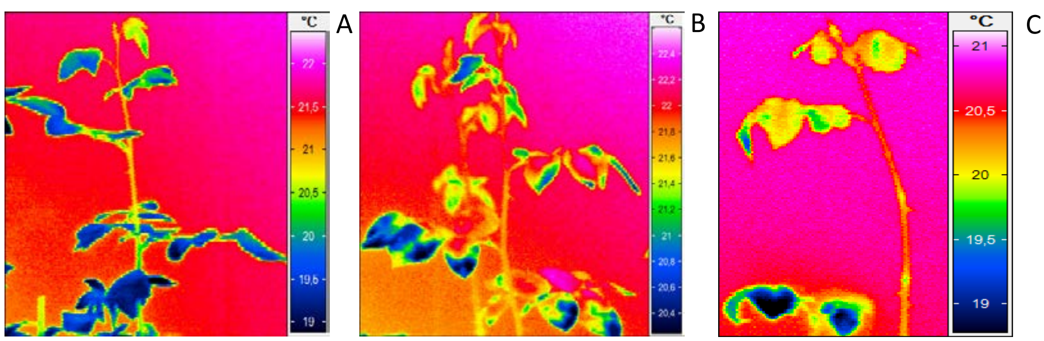

Figure 3. Effect of *Peronospora sparsa* infection on leaf temperature of rose cv. Elle® during pathogenesis visualized by IR thermography. A. Non-inoculated plant 4 days after inoculation (dai); B. Inoculated plant 4 dai; C. Inoculated plant 9 dai.

The MTD of inoculated leaves increased significantly over that of non-inoculated leaves one day before the presence of initial visual symptoms of the disease. The highest MTD value (1.23 K) was registered 4 dai and was related to an increase in leaf distortion and the presence of brown angular spots on leaves. In early stages of symptom development, the thermal profile was heterogeneous.

In contrast, non-inoculated leaves were characterized by homogeneous low temperature and low MTD over time (Fig. 4). In infected plants, MTD decreased progressively and coincided with the progress of the disease symptoms.

MTD has been reported as a parameter suitable for the detection of plant diseases by thermography (Oerke et al., 2006; Stoll et al., 2008; Wang et al., 2012). As suggested by Oerke and Steiner (2010), MTD is a highly sensitive parameter for early detection of plant pathogens. The obtained results agree with the previous reports and MTD becomes a suitable parameter for rose downy mildew detection by thermal imaging. In the current study, the use of thermography for the detection of primary foci of diseased plants at the commercial level seems to be suitable because of the unambiguous host plant response observed.

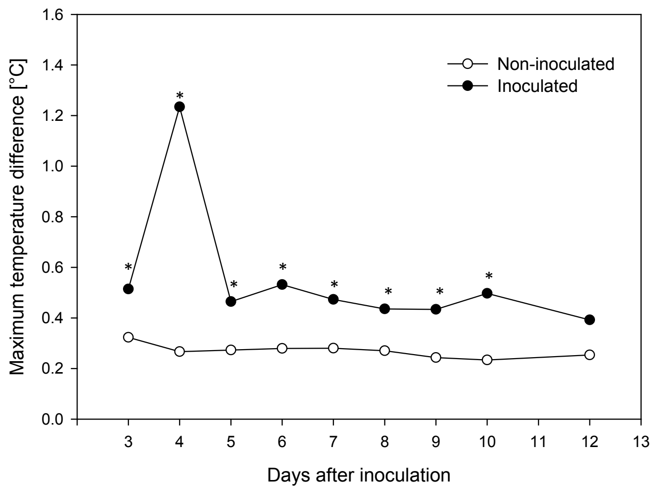

Figure 4. Effect of *Peronospora sparsa* infection on maximum temperature difference (MTD) of rose leaves cv. Elle®. Asterisks represent values significantly different from non-inoculated leaves (n = 12; t-test, $P < 0.05$).

CONCLUSION

Using thermal imaging the detection of *P. sparsa* infection was possible before the presence of symptoms of the disease. The dynamics of leaf temperature were associated with the development of the disease symptoms. Leaf colonization by *P. sparsa* was clearly associated with an increased leaf temperature throughout the pathogen development. Therefore, the use of thermography for the detection of primary foci of diseased plants at the commercial level seems to be suitable. This technique is a promising tool to be used in risk assessment programs of rose crops in commercial production systems.

LITERATURE

Aegerter, B.J., Nuñez, J.J. and Davis, R.M. 2002. Detection and management of downy mildew in rose rootstock. Plant Dis. 86:1336-1368.

Alfieri, S.A. 1968. Downy mildew of rose caused by *Peronospora sparsa* Berk. Florida Department of Agriculture Division of Plant Industry. Plant Pathol. Circular No. 66.

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

Clark, J.S.C., and P.T.N. Spencer-Phillips. 2004. The compatible interactions in downy mildew. Pages: 1–34. *In* P.T.N. Spencer-Phillips and M.J. Jeger (ed.), Advances in Downy Mildew Research Vol. 2. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Francis, S.M. 1981. *Peronospora sparsa.* Commonwealth Mycologycal Institute Descriptions of Pathogenic Fungi and Bacteria. N°690. CIM, Kew, Surrey, England.

Grant, O.M., Chaves, M.M. and Jones, H.G. 2006. Optimizing thermal imaging as a technique for detecting stomatal closure induced by drought stress under greenhouse conditions. Physiol. Plantarum 127:507-18.

Gullino, M.L., and Garibaldi, A. 1996. Diseases of rose: evolution of a problem and new approaches for their control. Acta Hortic. 424:195-201.

Horst, K. 1983. Compendium of Rose Diseases. The American Phytopathological Society, St. Paul, USA. 50 p.

Jones, H.G. 2004. Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. Adv. Bot. Res. 41:107-163.

Karlik, J.F., and Tjosvold S.A. 2003. Integrated pest management. Pages 466-473. *In* A. Roberts, T. Debener and S. Gudin, (ed.), Encyclopedia of Rose Science. Academic Press, London, UK.

Lindenthal, M., Steiner, U., Dehne, H.-W., and Oerke, E.-C. 2005. Effect of downy mildew development on transpiration of cucumber leaves visualized by digital infrared thermography. Phytopathology 95:233-240.

Oerke, E.-C., Fröhling, P. and Steiner, U. 2011. Thermographic assessment of scab disease on apple leaves. Precision Agric. 12:699-715.

Oerke, E.-C. and Steiner, U. 2010. Potential of digital termography for disease control. Pages: 168-183. *In* Oerke, E.-C., Gerhards, R., Menz, G. and Sikora, R.A. (ed.), Precision Crop Protection - the Challenge and Use of Heterogeneity. 1st Edition, Springer. 441 p.

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

Stoll, M., Schultz, H.M., Baecker, G. and Berkelmann-Loehnertz, B. 2008. Early pathogen detection under different water status and the assessment of spray application in vineyards through the use of thermal imagery. Precision Agric. 9:407–417.

Suárez, J.R. 1999. Problemática actual del mildeo velloso en rosa. *In* VIII Congreso Acopaflor - Asocolflores. Bogota, Colombia.

Wang, M., Ling, N., Dong, X., Zhu, Y., Shen, Q., and Guo, S. 2012. Thermographic visualization of leaf response in cucumber plants infected with the soil-borne pathogen *Fusarium oxysporum* f. sp. *cucumerinum*. Plant Physiol. Bioch. 61:153–61.

Wheeler, B.E.J. 1981. Downy mildews of ornamentals. Pages: 461-451. *In* D.M. Spencer (ed.), The Downy Mildews. Academic Press, London, UK.

Xu X.-M., and Pettitt, T. 2003. Rose downy mildew. Pages: 154-158. *In* A. Roberts, T. Debener and S. Gudin, (ed.), Encyclopedia of Rose Science. Academic Press, London, UK.