THERMOGRAPHY AS SENSOR FOR DOWNY MILDEW ON ROSES

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ABSTRACT

Downy mildew caused by *Peronospora sparsa* is one of the most important diseases affecting cut roses under glass in the tropics. Under favorable environmental conditions, rapid epidemics of the pathogen hard to control may cause the total loss of susceptible rose cultivars in a plantation. Disease detection is based on close inspection of plants to identify affected areas in the greenhouse. However, this method is time consuming, expensive and in the majority of cases not suitable for the detection of initial disease symptoms. In spite of this, only limited research has been conducted to find alternative methods for the early detection of *P. sparsa*. Non-invasive sensor techniques like infrared thermography have been reported to offer a high potential for monitoring plant-pathogen interactions. The aim of this study was to investigate the ability of thermography for the detection of downy mildew symptoms on roses under controlled conditions as the first step to its subsequent use under commercial conditions. Leaves and detached full stems of two rose cultivars were inoculated and the development of the infection was followed by visual inspection and with thermal imaging using a camera system with 0.03 K thermal resolution. Disease symptoms on the susceptible cultivars were observed 6 days post inoculation (dpi) by visual inspection while in themograms the presence of the pathogen was detected 3 dpi. Furthermore, differences in the temperature of leaves were observed during the spread of the disease throughout the evaluation period. The results indicate that the use of thermography may be an alternative tool to detect downy mildew infection on roses in early stages also under production conditions.

Keywords: Sensing, early detection, *Peronospora sparsa*, *Rosa* sp.

INTRODUCTION

Downy mildews are considered one of the most devastating plant diseases, which under suitable environmental conditions may lead to total loss of the crop. Its importance and difficult management is related to development of epidemics in affected crops, in short periods of time that may vary within four and seven days after appearance of the first plants affected by the disease (Hildebrand and Sutton, 1985; Urban et al., 2007). Downy mildew symptoms in rose caused by

Peronospora sparsa are found on leaves, stems, peduncles, calices, and petals; commonly the infection is limited to young apexes (Aegerter et al., 2002). The capacity of downy mildews to form systemic infections in many plants is reported. Symptomless invasion of the stem tissues resulted in systemic infection of *Pisum sativum* by *Peronospora viciae*, indicating that some apical infection may originate from leaf lesions (Taylor et al., 1990). Williamson et al. (1995) observed hyphae of *Peronospora rubi* entering the veins and petioles and spreading to the cortical tissues of the stem of *Rubus* spp. Aegerter et al. (2002), demonstrated by PCR and microscopy the permanence of *P. sparsa* infections in stems, roots, and crown of rose plants with no apparent symptoms.

Non-destructive imaging techniques as reflectance measuring, fluorescence or temperature of leaves have been used for monitoring the physiological reaction of plants to pathogen attack by several authors, e.g. Omasa et al. (1983), Chaerle and Ven der Staeten (2000) and Riera et al. (2005). Non-invasive sensor techniques like infrared thermography have been reported to offer a high potential for monitoring plant-pathogen interactions. This technology, based on the principle that transpiration of water through stomata, cools leaves, and hence stomatal closure results in decrease temperature (Grant et al., 2006) is a promising alternative to study biotic factors causing stress in plants. Perturbation of transpiration may be used as cues for the development of plant diseases affecting stomatal aperture and functionality of cuticule integrity (Oerke and Steiner, 2010). Pathogens can influence stomatal aperture by interposing water transport or by releasing specific compounds that induce plant responses (Jones, 2004, Chaerle et al., 2004).

Infrared thermography visualized the establishment of *Erwinia amylovora* harpin-induced hypersensitive response (HR) in *Nicotiana sylvestris* leaves (Boccara et al., 2001). Before visual symptoms appeared, thermography permitted to monitor an increase in temperature after infection of resistant tobacco by tobacco mosaic virus (Chaerle et al., 2002). Chaerle et al. (2004) pointed out that knowledge of disease signatures for different plant–pathogen interactions could allow early identification of emerging biotic stresses in crops, after monitored spots of lower temperature with thermography, in the system sugar beet – *Cercospora beticola* in marked contrast with observations on TMV-infection in tobacco. In cucumber leaves, before visible symptoms of downy mildew caused by *Pseudoperonospora cubensis* appeared, the maximum temperature difference within thermograms allowed the discrimination between healthy and infected leaves (Lindenthal, et al., 2005). Moreover, these results showed that infra-red thermography can be successfully applied for pre-symptomatic detection of downy mildew attack in cucumber (Lindenthal et al., 2005; Oerke et al., 2006). The interaction between *Plasmopara viticola* and grapevine leaves was detected thermographically on 3 or 4 days after inoculation, before any visual symptoms occurred. In addition, the downy mildew pathogenesis resulted in a considerable heterogeneity in spatial and temporal variation of leaf temperature (Stoll et al., 2008).

Up to now, rose downy mildew detection is based on close inspection of plants to identify affected areas in the greenhouse. However, this method is time consuming, expensive and in most cases not suitable for the detection of initial disease symptoms. In addition, the occurrence of the disease in symptomless

tissues or even in symptomless plants makes the evaluation of the process more complicated. In spite of this, only limited research has been conducted to find alternative methods for the early detection of *P. sparsa*. Therefore, it is necessary to improve the diagnostic methods to implement timely disease control. The use of imaging technologies allows the possibility of new approaches to study infection processes of detached organs and whole plants. The aim of this study was to assess the infection process of *P. sparsa* in detached leaves and shoots in a non-destructive way and to investigate the ability of thermography for the detection of downy mildew symptoms on roses as the first step to its subsequent use under commercial conditions.

MATERIALS AND METHODS

Detached rose leaves of the susceptible cultivars cv. Elle® and cv. Sweetness® kept under greenhouse conditions where used to evaluate the process infection of the pathogen with thermography. Due to the fact that previous results demonstrated that the fastest spread of the pathogen was observed in young leaves, this condition of maturity was used in the study. The rose leaves, composed of five leaflets were placed with the adaxial side in touch with wet filter paper in Petri dishes. For the inoculation an isolate from Colombia kept under controlled condition in the laboratory was used. The isolate was maintained on rose leaflets since *P. sparsa* is an obligate parasite. Two drops of 25 □ of a suspension of 1 x 10^4 sporangia per ml. of the pathogen were placed in the center of the second right leaflet of the leaf and then distributed uniformly using a soft brush to cover a stripe of 0.5 cm. After inoculation the leaves in the Petri dishes were kept under 18°C/16°C day/night temperature and 16 hours of light in a growth chamber. Two days after inoculation (dpi), daily evaluations were carried out on inoculated and noninoculated leaves visually and under the stereoscope to follow the development of the pathogen through the presence of sporulation and with thermography. The plant material under evaluation was adapted to the condition of the room (25 ± 2°C, 40 ± 5% RH) for at least 60 minutes before thermographic evaluations. As detached leaves adapt to the temperature of water they are floating on, the leaves were placed on a paper towel for 10 second and after transfer to a humid chamber for 20 minutes to continue the drying process avoiding the desiccation of the leaves and in consequence dehydration of the pathogen. After this time period thermal imaging were taken from a distance of 30 cm from the leaves under evaluation.

To assess the pathogen infection on shoots, young plant material of rose cv. Elle® was harvested from the plants and placed in 50 ml. Eppendorf® tubes with distilled sterile water and sealed with Parafilm® to hold the shoot in the center of the tube. Apical leaflets of every shoot were inoculated with a suspension of *P. sparsa* of 1 x 10^4 sporangia per milliliter. Then inoculated and noninoculated shoots were kept for a period of 48 hours at 10°C in high relative humidity conditions to ensure pathogen infection and were then maintained at 18°C/16°C day/night temperature in a growth chamber. The development of the infection in the shoots was followed by visual inspection and by thermal imaging. Visual

assessments were made daily to establish the first appearance of disease symptoms at the site of inoculation and on neighboring areas. The inoculated and noninoculated shoots were adapted to the condition of the room (25 ± 2°C, 40 ± 5% RH) at least for 60 minutes before thermal images were taken. Thermograms were registered at a distance of 50 cm from the plant material under study.

Thermographic 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 □n and 1.5 m radiar
(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.

Stomatal aperture of infected young leaf disks of the cv. Elle® was evaluated 24 hours post inoculation (hpi) and every two days during 17 days. After inoculation the leaf discs were kept in Petri dishes under 18°C/16°C day/night temperature and 16 hours of light in a growth chamber. Imprints of the abaxial side of inoculated and noninoculated leaf discs were taken using nail polish and peeled off with transparent adhesive tape and fixed on a glass slide for evaluations (Lindenthal, et al., 2005). The width of stomatal aperture was measured using a photomicroscope (Leitz DMRB; Leica, Wetzlar, Germany) and the software Diskus 4.2 (Hilgers, Königswinter, Germany). The average of stomatal aperture was calculated using 50 stomata from five replicates.

RESULTS AND DISCUSION

Imaging of downy mildew on leaves. Changes in infected rose leaves temperature over the time were detected with thermography after localized inoculation of the pathogen. In addition, the same pattern of temperature was observed on the adaxial and abaxial side of the infected leaves. This observation shows that thermal effects of infection and development of *P. sparsa* can be detected with thermography on both sides of the leaves. Similar observations were obtained by Kümmerlen et al. (1999) with apple scab caused by *Venturia inaequalis* where symptoms on one leaf side reduced the temperature of both, adaxial and abaxial leaf surface.

High temperatures at the site of inoculation were detected 3 dpi and this condition was observed until 11 dpi in cv. Elle® and 13 dpi in cv. Sweetness®. After this time, a noticeable decrease in temperature was registered in infected tissue of both cultivars (Fig.1). Similar results were observed by Lindenthal et al. (2005) who detected changes in leaf temperatures during downy mildew development. The increase of infected leaflet temperature in the first stages of *P. sparsa* development are in accordance with the effect observed of *Pseudoperonospora cubensis* the downy mildew of cucumber that caused an increase in temperature of infected leaves at early stages of the disease (Lindenthal et al. 2005, Oerke et al., 2006).

Differences in the development of the pathogen in the two cultivars under study were observed with thermal imaging. In cv. Elle® high temperatures at the inoculated site were observed 6dpi. In inoculated leaflets of cv. Sweetness® showed high temperatures significantly different from noninoculated leaflets 2 dpi, and 6 dpi until 13 dpi. A decrease in temperature of the inoculated leaflet was observed 12 dpi in cv. Elle ® and 15 dpi in cv. Sweetness® (Fig. 1).

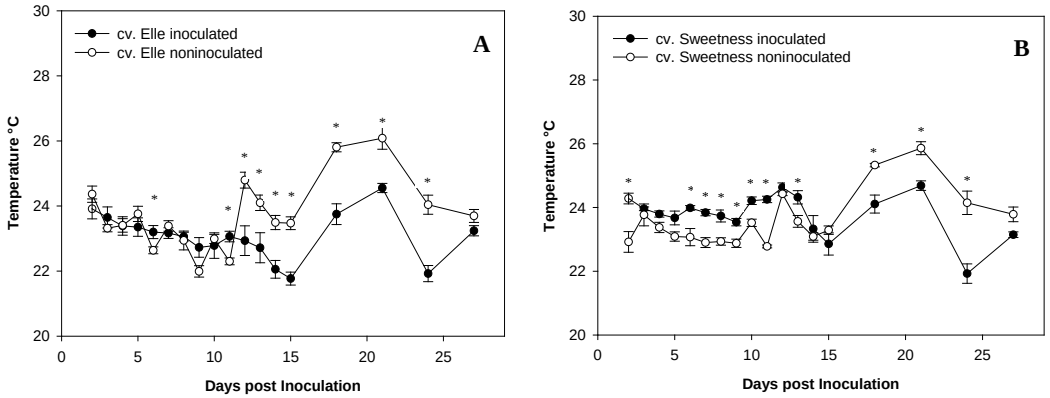

Figure 1. Effect of *Peronospora sparsa* infection on the temperature of inoculated leaflets of rose cv. Elle® (A) and cv. Sweetness® (B) under controlled conditions. Error bars represent standard error. Asterisk (*) indicates values significantly different (*t* test, $P \leq 0{,}050$)

Temperatures registered of the inoculated leaflet (No. 3) and neighboring leaflets (Nos. 1, 2, 3 and 4) are presented in Fig. 2. In both cultivars high temperatures were detected 6 dpi on the inoculated leaflet. In cv. Elle® 14 dpi a significantly decrease in temperature of neighboring leaflets of the inoculated site compared with the noninoculated leaflets were observed. Moreover, thermograms of this cultivar taken 24 dpi detected a significantly low temperature in all the leaflets of the leave. On the contrary, on neighboring leaflets of the inoculated site of cv. Sweetness® high temperatures were registered 14 dpi and low temperatures caused by the infection of *P. sparsa* were not detected 24 dpi except for the inoculated leaflet. In terms of the development of the pathogen, in cv. Elle® sporulation of *P. sparsa* was observed 11 dpi on the apical leaflet and on the leaflet opposite to the inoculated site and 12 dpi on leaflets No. 3 and No. 4. In cv. Sweetness® sporulation of the pathogen on the apical leaflet was observed 15 dpi, 11 dpi on leaflet No. 2 and 13 dpi in leaflets No. 4 and No. 5.

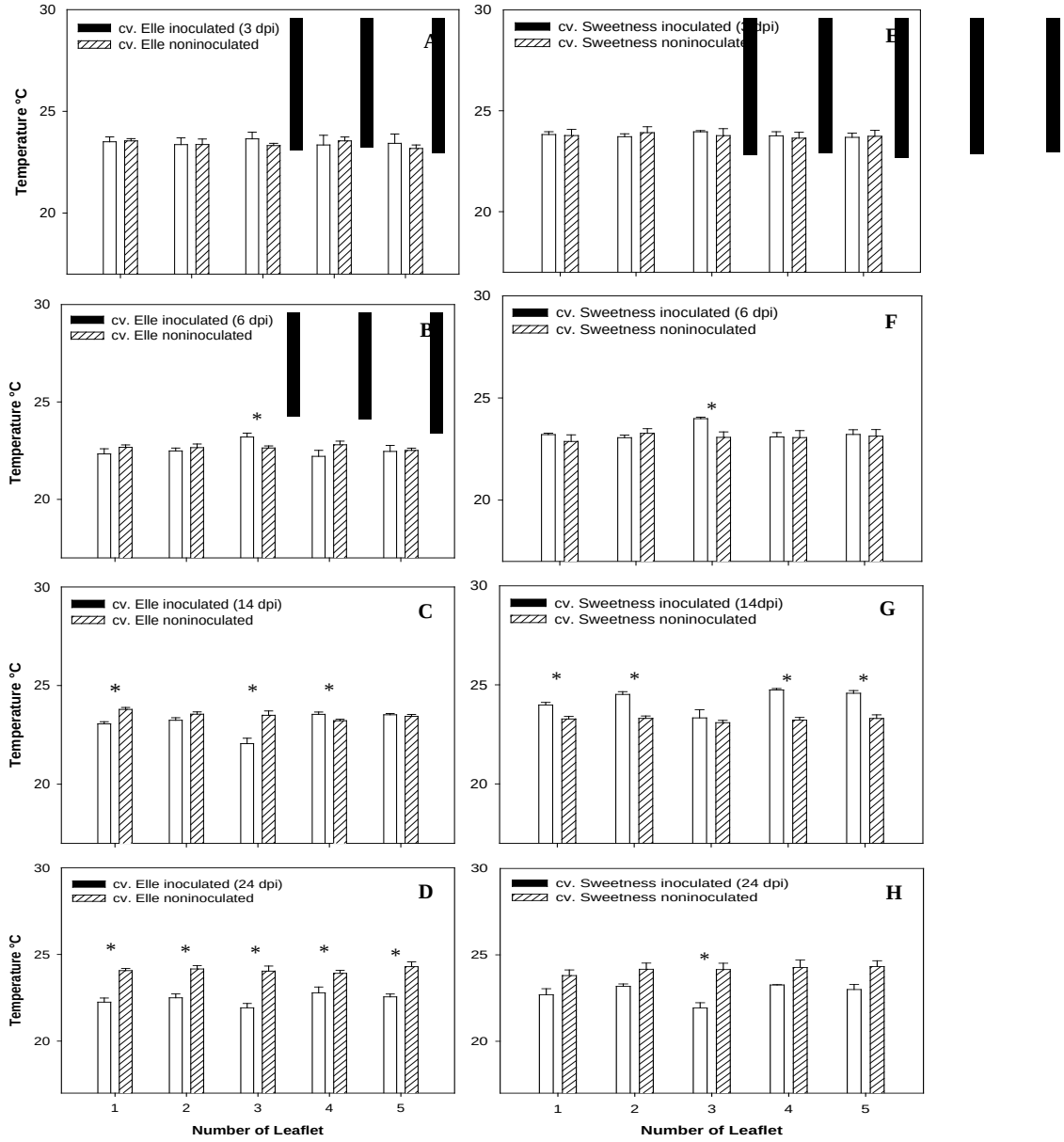

Figure 2. Effect of *Peronospora sparsa* infection on the temperature of rose leaves cv. Elle® (A to D) and Sweetness® (E to H) over the time. Numbering of leaflets in x- axis: number 1 corresponds to the apical leaflet, leaflets number 2 and 3 are middle leaflets where leaflet number 3 was inoculated. Leaflets 4 and 5 are proximal stem pair of leaflets. A. and E. 3 dpi B. and F. 6 dpi C. and G. 10 dpi D. and H. 24 dpi. Error bars represent standard error. Asterisk (*) indicates values significantly different (*t* test, $P \leq 0{,}050$)

The differences detected with thermal imaging among the two cultivars evaluated after the infection of *P. sparsa* shows the value of this technique in the study of *P. sparsa* – rose interaction. Oerke et al. (2011) observed with thermography differences in apple scab severity due to the resistance of the leaves and the aggressiveness of the isolates of the pathogen. These results demonstrate

that infrared thermography is a useful technique for the study of plant diseases from different approaches.

The results obtained indicate that the temperature associated with the infection of *P. sparsa* is dynamic over the time and is related with the progress of the pathogen within the rose tissues. Downy mildew infection is characterized by sporangia germination, penetration of epidermal cell walls to enter intercellular spaces of the mesophyll tissue and finally the production of sporangiophores through the stomata of the leaves (Ingram and Irene, 1971, Williamson et al., 1995, Aegerter et al., 2002). At the inoculation site first sporangiophores with sporangia of *P. sparsa* were observed 6 dpi in both cultivars. Sporulation of the pathogen on the surrounding areas of the inoculated site was observed 10 dpi in cv. Elle® and 9 dpi in cv. Sweetness® and the inoculated leaflets were completely cover by a dense sporulation 12 dpi and brown tissue became visible 15 dpi (Fig. 3).

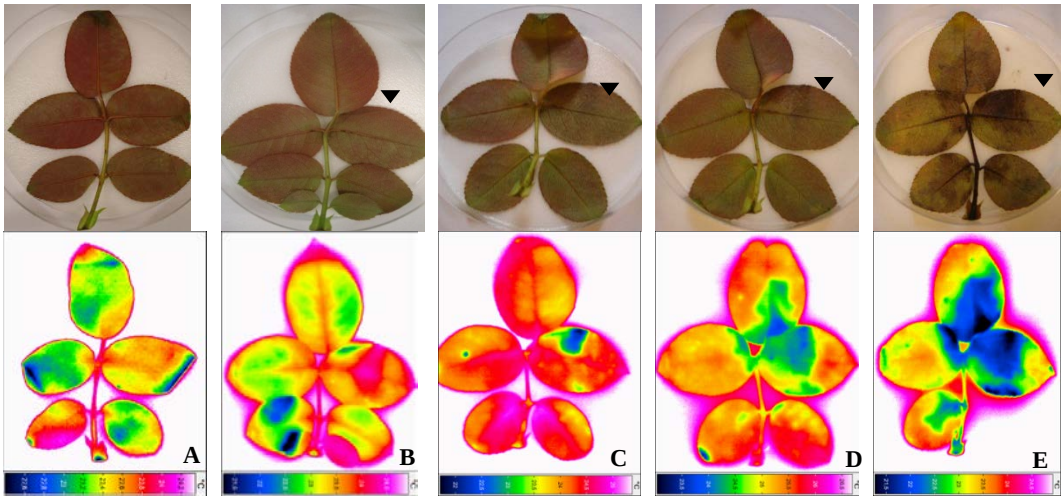

Figure 3. Development of *Peronospora sparsa* from a localized inoculation site (▼) on detached leaves of cv. Elle® at different days post inoculation (dpi). RGB reflectance images and thermograms show the development of the infection. A. Noninoculated leaf. B. 3 dpi C. 13dpi D. 21dpi and E. 27 dpi

In this study was observed that *P. sparsa* can establish spreading infections on roses that are not limited to the initial inoculation site. Visual inspection of the inoculated leaves showed as a result of pathogen spread, sporulation of *P. sparsa* in neighboring leaflets. In terms of the infected area expansion, thermograms also showed the pathogen spreading from a local site of inoculation (Fig. 3). The initial high temperature spots expanded the first days after the inoculation and later low temperatures of infected leaves were registered following the same pattern of presence of dense sporulation of *P. sparsa* associated with the development of areas of the tissue brown color.

Imaging of *Peronospora sparsa* on rose shoots. Thermograms of infected rose shoots detected significant differences in temperature of inoculated apical leaflets of rose cv. Elle® compared with noninoculated leaflets. First an increase of temperature of the inoculated leaflets 7 dpi was observed and 10 dpi a significant lower temperature of the infected leaflets was registered (Fig. 4).

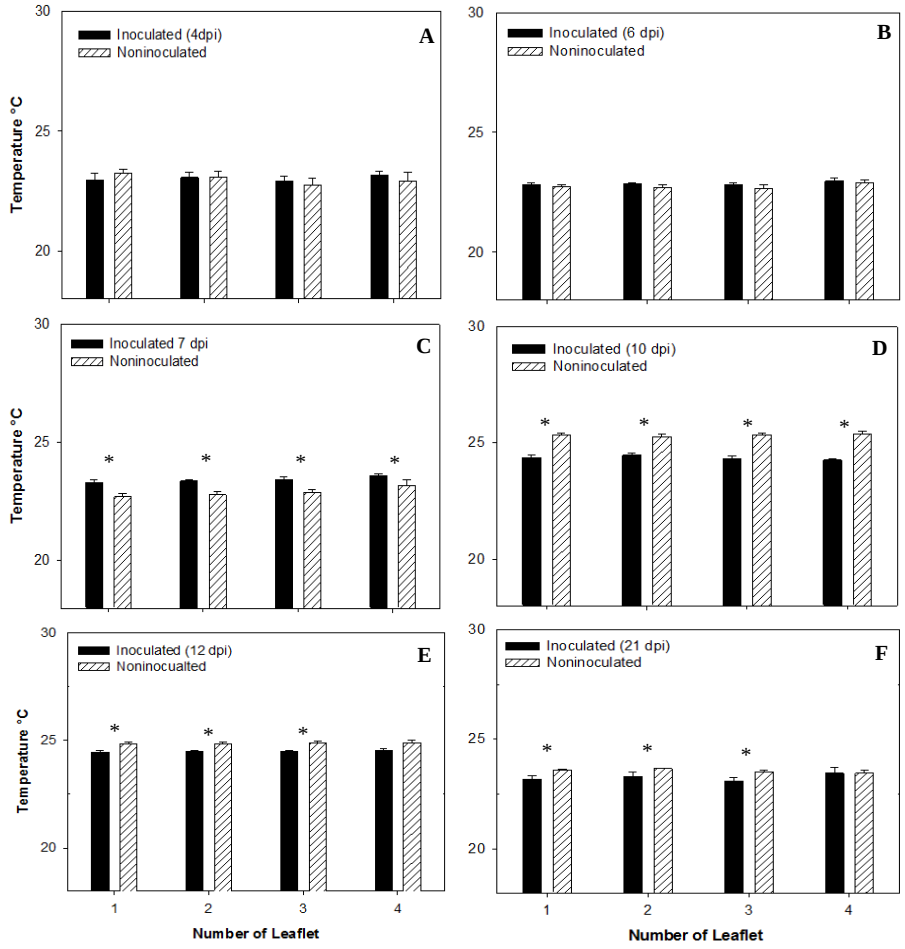

Figure 4. Effect of *Peronospora sparsa* infection on temperature of rose apical leaflets of shoots cv. Elle® over the time. A. 4 dpi B. 6 dpi C. 7 dpi D. 10 dpi E. 12 dpi. F. 21 dpi. Error bars represent standard error. Asterisk (*) indicates values significantly different (*t* test, $P \leq 0{,}050$)

Previous studies with other downy mildews have shown early detection of the pathogen infection with infrared thermography. In this study symptoms of the diseased were not observed before 10 dpi. Nevertheless at this period of time infrared thermography detected differences in the temperature between inoculated and noninoculated leaflets. First symptoms of downy mildew such as dull leaflets and light brown spots were observed 11 dpi and typical irregular spots, purple color to dark brown, and chlorosis were evident 12 dpi (Fig. 5). Severe symptoms of the disease were observed at the time that a decrease in leaflet temperature was detected with thermal imaging. Stoll et al. (2008) detected with thermography

infection of *Plasmopara viticola* before the appearance of visual symptoms. In *P. cubensis* - cucumber interaction, cold areas of the infected tissue were registered one day before of appearance of disease symptoms (Lindenthal et al., 2005).

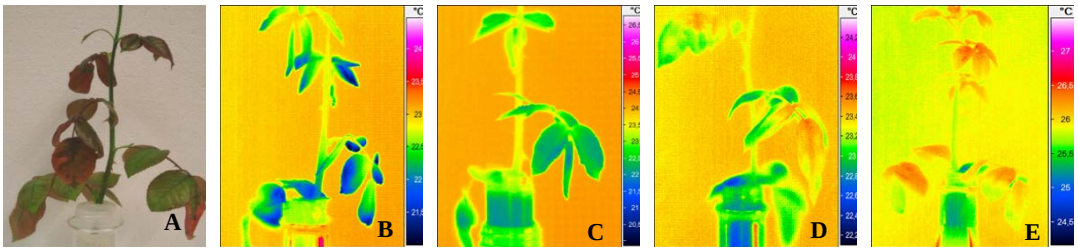

Figure 5. Effect of *Peronospora sparsa* development from localized site inoculation on temperature of apical leaflets of rose shoots cv. Elle®. A. Digital image of an infected shoot with symptoms of the disease 15 dpi B. Noninoculated shoot 7 days C. 4 dpi D.7 dpi E.12 dpi

Sporulation of *P. sparsa* was not observed on the infected leaflets due to the fact that the inoculated and noninoculated shoots were kept in a growth chamber with a relative humidity under 85%. Pathogens causing downy mildews in general, require high relative humidity to sporulation as reported by Hildebrand and Sutton, (1985). Nevertheless a decrease in temperature of infected leaflets of the rose shoots was observed preceded by a period of high temperature similar to the temperature dynamic observed with thermography of detached infected leaves. In rose shoots, under the conditions of this study the development of the pathogen took more time. As a result, changes in temperature of infected leaflets were observed later in comparison with the changes noticed with thermography in detached leaflets where the pathogen had ideal condition for its development.

Stomatal aperture of leaves infected by *Peronospora sparsa.* Diseases that affect stomata regulation may produce modifications in the transpiration before the presence of the symptoms, others when visible symptoms appear and some affect cuticular transpiration when the tissue is severely affected (Oerke and Steiner, 2010). The stomatal opening observed of infected leaves discs compared with the healthy ones indicated that the infection of *P. sparsa* affects the behavior of the stomata aperture in rose. At the beginning of the infection statistical differences in stomatal aperture were found 3dpi however hypha of the pathogen were observed under the epidermis and coming through the stomata of leaves discs 6 dpi to produce the first sporangiophores and sporangia. Significant differences in stomatal opening of the inoculated discs in comparison with the noninoculated were found 8 and 11 dpi were the stomata of infected leaves showed less aperture. Open stomata on infected rose leaves were observed 13, 15 and 17 dpi that coincides with advanced stages of *P. sparsa* infection with a dense sporulation and the presence of brownish tissue (Fig. 6).

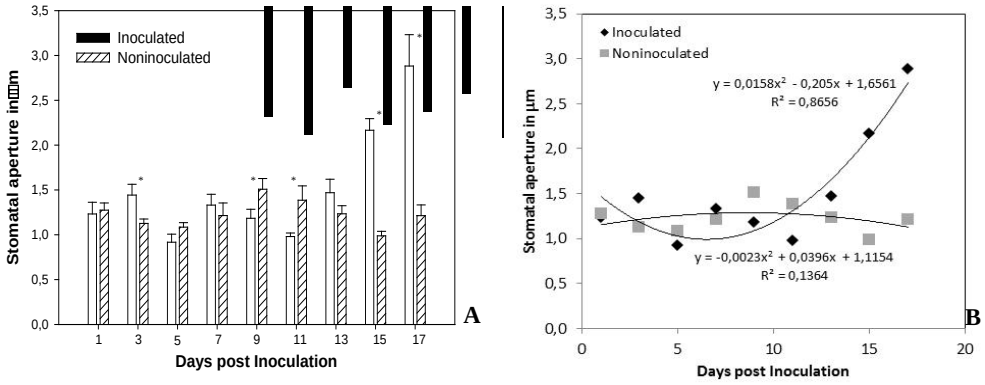

Figure 6. Stomatal aperture of rose leaf discs noninfected and infected with *Peronospora sparsa* during the infection process. Error bars represent standard error. Asterisk (*) indicates values significantly different (*t* test, $P \leq 0{,}050$)

The effect of *P. sparsa* infection on the stomatal aperture of rose leaves discs may be related with the extensive colonization by intercellular mycelium of the pathogen and the profuse sporulation through the stomata of the leaves that can affect its structure and function (Fig. 7). A similar unnatural stomatal opening associated with loss of membrane integrity was observed by Lindenthal et al. (2005) in downy mildew of cucumber. Likewise, infection on potato by *Phytophthora infestans* induced stomata to open more than normal under different light conditions (Farrel et al., 1969).

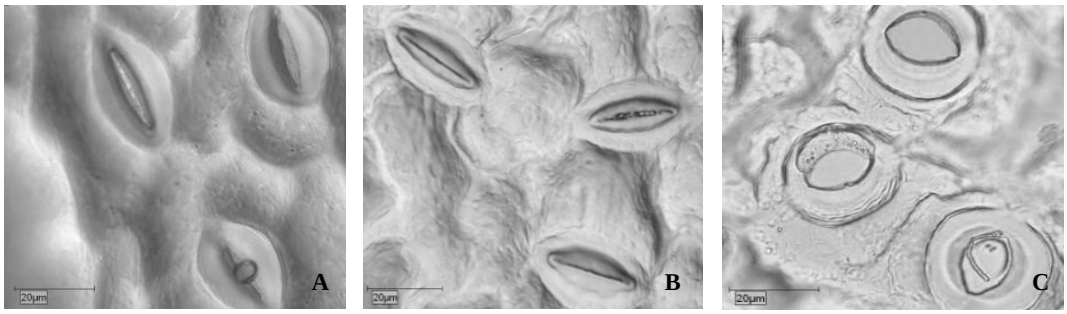

Figure 7. Stomata of rose leaves of cv. Elle® infected by *Peronospora sparsa*. A. Stomata of healthy leaf B. Infected leaf 1 dpi C. Infected leaf 15 dpi

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 this study, a decrease in infected leaflets temperature was observed associated to the presence of severe stages of development of the disease not only in complete leaves of two susceptible rose cultivars but also in detached shoots.

CONCLUSION

In this study, detection of *P. sparsa* infection before the presence of symptoms of the disease was possible using thermal imaging. In addition, the dynamic of leaf temperature associated with the development of the pathogen within rose tissue was visualized and differences between rose cultivars in terms of development of the infection were observed. The results indicate that the use of thermography may be a suitable alternative tool to detect downy mildew infection on roses in early stages nevertheless the technique has to be evaluated in whole plants and under production conditions. In the future, this technique would help to enhance efficiency in crop management at commercial crops.

LITERATURE

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

Boccara, M., Boue, C., De Paepe R. and Boccara, A.C. Early events in plant hypersensitive response leaves revealed by IR thermography, Proc.SPIE 4434, 183 (2001) http://dx.doi.org/10.1117/12.446675

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 and Cell Physiology 45: 887-896. SCI 3.26

Chaerle, L., vande Ven, M., Valcke, R. and Van Der Straeten, D. 2002. Visualisation of early stress responses in plant leaves. Thermosense XXIV, Proceedings of SPIE Vol. 4710, p417-423.

Chaerle L. and Van Der Straeten, D. 2000. Imaging techniques and the early detection of plant stress. Trends Plant Sci 5: 495–501

Farrel, G.M., Preece, T.F. and Wren, M. 1969. Effects of infection by *Phytophthora infestans* (Mont.) de Bary in the stomata of potato leaves. Ann. Appl. Biol. 63:265-275.

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 Physiologia Plantarum 127:507– 18.

Hildebrand P.D. and Sutton J.C. 1985. Environmental water in relation to *Peronospora destructor* and related pathogens. Canadian Journal of Plant Pathology. Volume 7, Issue 3

Ingram, D.S. and J. Irene. 1971. The Growth of *Peronospora farinosa* f. sp. *betae* and Sugar Beet Callus Tissues in Dual Culture. Journal of General Microbiology. 69: 211-220

Jones, H.G. 2004. Application of thermal imaging and infrared sensing in plant physiology and ecophysiology. Advances in Botanical Research incorporating Advances in Plant Pathology. 41, 107–163.

Kümmerlen, B., Dauwe, S., Schmundt, D. and Schurr, U. 1999.Termography to measure water relations of plant leaves. In: Jähne B (ed) Handbook of computer vision and application, Academic Press, London. p. 636-672.

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.

Omasa, K., Hashimoto, Y. and Aiga, I. 1983. Observation of stomatal movements of intact plants using an image instrumentation system with a light microscope. Plant Cell Physiol 24: 281–288

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

Oerke, E.-C. and Steriner, U. 2010. Potential of digital termography for disease control p. 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, XXIV. 441 p.

Oerke, E.-C., Steiner, U., Dehne, H.W., & Lindenthal, M. 2006. Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions. Journal of Experimental Botany, 57, 2121–2132

Riera, M., Valon, C., Fenzi, F., Giraudat, J. and Leung, J. 2005. The genetics of adaptive responses to drought stress: abscisic acid-dependent and abscisic acid-independent signaling components. Physiol. Plant 123: 111–119

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

Taylor, P.N., Lewis, B.G. and Mattews, P. 1990. Factors affecting systemic infection of *Pisum sat ivum* by *Peronospora viciae.* Mycol. Res. 94 (2): 179-181

Urban, J., Lebeda, A. and Pejchar, M. 2007. Deferential sensitivity to fungicides in Czech populations of cucumber downy mildew (*Pseudoperonospora cubensis*) In: Advances in Downy Mildew Research, Vol 3. 2007. A. Lebeda and P. T.N. Spencer- Phillips (Eds.) ISBN 80-8663-19-4. P. 251 – 255.

Williamson, B., Bresse, W.A. and Shattock, R.C. 1995. A histological study of downy mildew *(Peuonospoua rubi)* infection of leaves, flowers and developing fruits of Tummelberry and other *Rubus* spp. Mycol. Res. 99 (11): 1311-1316.