RESEARCH PAPER

Thermal imaging of cucumber leaves affected by downy mildew and environmental conditions

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Abstract

Pathogenesis of Pseudoperonospora cubensis causing downy mildew of cucumber resulted in changes in the metabolic processes within cucumber leaves including the transpiration rate. Due to the negative correlation between transpiration rate and leaf temperature, digital infrared thermography permitted a non-invasive monitoring and an indirect visualization of downy mildew development. Depending on the stage of pathogenesis and the topology of chloroses and necroses, infection resulted in a typical temperature pattern. Spatial heterogeneity of the leaf temperature could be quantified by the maximum temperature difference (MTD) within a leaf. The MTD increased during pathogenesis with the formation of necrotic tissue and was related to disease severity as described by linear and quadratic regression curves. Under controlled conditions, changes in temperature of infected leaves allowed the discrimination between healthy and infected areas in thermograms, even before visible symptoms of downy mildew appeared. Environmental conditions during thermographic measurement, in particular air temperature and humidity, as well as water content and age of the leaf influenced the temperature of its surface. Conditions enhancing the transpiration rate facilitated the detection of changes in leaf temperature of infected leaves at early stages of infection. As modified by environmental conditions, MTD alone is not suitable for the quantification of downy mildew severity in the field.

Key words: Cucumis sativus, digital thermography, leaf temperature, Pseudoperonospora cubensis, transpiration.

Introduction

Leaf temperature of plants is the result of external and internal (physiological) factors. The environmental factors solar radiation, air temperature, and relative humidity (RH), and the water status of the shoot tissue determine the temperature of plants via stomatal transpiration. There is a correlation between leaf temperature and water status, as water is the primary source of infrared absorption in plant tissue (Kümmerlen et al., 1999).

In addition to water supply and overall metabolic activity regulated by environmental conditions, pathogenic organisms may affect both cuticular and stomatal conductance of plant tissue, resulting in significant modifications in leaf temperature. As leaf temperature may be measured remotely and with high spatial resolution, digital infrared thermography may have the potential for the identification of management zones in disease control.

The detection of modifications in plants or canopies associated with low disease severity in the early stages of disease epidemiology is crucial for the targeted, site-specific or on-demand application of fungicides in integrated disease control. The sensing of ethylene associated with tissue damage from pathogens (Boller, 1983) and optical methods assessing the reflection and fluorescence characteristics of plants associated with photosynthetic activity (Scholes and Rolfe, 1996; Coops et al., 2003; Laudien et al., 2004; Franke et al., 2005) are some approaches. Thermography allows the quantitative analysis of spatial and dynamic physiological information on the plant status (Jones, 2004).

In plant biology, infrared thermography is used to study spatial variability of stomatal conductance (Jones, 1999a; Omasa and Takayama, 2003; Prytz et al., 2003), to schedule irrigation (Gebhardt, 1990; Jones 1999b), for monitoring of ice-nucleation or temperature stress in plants (Wisniewski et al., 1997; Yang et al., 2003), to screen for
mutants with altered stomatal control (Merlot et al., 2002; Wang et al., 2004), and for assessment of plant–pathogen interaction by monitoring patterns of surface leaf temperature (Chaerle et al., 1999).

For remote detection, identification, and quantification of plant diseases and associated pathogens, sensors have to be sensitive to physiological disorders associated with fungal attack and disease resulting from pathogen attack and tissue colonization. In contrast to weeds which can be remotely detected and identified in crops according to their macroscopic shape early after emergence (Gerhards and Christensen, 2003), micro-organisms causing plant diseases may be detected only by their effect on plant tissue; visible symptoms often appear only after latent colonization of the plant tissue. Digital infrared thermography has been shown in previous studies to be a useful tool for the presymptomatic detection of cucumber downy mildew caused by Pseudoperonospora cubensis (Berk. et Curt.) Rostovzev (Lindenthal et al., 2005; Oerke et al., 2005). The maximum temperature difference (MTD) within a leaf or a canopy turned out to be suitable for the differentiation of infected and non-infected tissue under controlled conditions.

Downy mildew of cucurbits is a devastating disease, especially in temperate regions of the world (Palti and Cohen, 1980; Lebeda and Schwinn, 1994) where humid conditions favour disease spread; infection by zoospores requires free water on the lower leaf surface for at least 2 h, and production of zoosporangia in the dark occurs at an RH of >90% for at least 6 h (Cohen et al., 1971; Cohen, 1977; Zitter et al., 1996). First symptoms on leaves are small, slightly chlorotic to bright yellow areas on the upper surface without loss of vitality in plant cells (Spencer, 1981). Lesions expand with time and may remain chlorotic or yellow or, depending on environmental conditions, become necrotic and brown. Further development of lesions results in the necrotization of progressively larger leaf areas, and in a few days the entire leaf may be destroyed.

The chronological and spatial dynamics in producing disease symptoms seem to be rather limited to downy mildew pathogens which have to colonize the new healthy leaf areas rapidly as—despite being biotrophic—the pathogens damage the colonized plant tissue (Spencer, 1981). Water loss from infected leaf areas can increase due to destruction of the leaf cuticle (Bassanezi et al., 2002), increased permeability of leaf cell membranes (Chaerle et al., 2001), or inhibition of stomatal closure (Smith et al., 1986; Felle et al., 2004). Reduction of transpiration may result from stomatal closure (Chaerle et al., 2001), obstruction of xylem elements and stomata (Wright et al., 2000; Bassanezi et al., 2002), and defoliation.

A negative correlation between transpiration rate and leaf temperature has been shown by Inoue et al. (1990). An increase in leaf temperature due to restricted water supply of the shoot has been described for diseases caused by root rot pathogens or wilt pathogens (Pinter et al., 1979; Nilsson, 1985a, 1995; Lili et al., 1991; Chaerle and van der Straeten, 2001). Necrotrophic leaf pathogens such as Pyrenophora spp. and Pseudomonas syringae as well as Phytophthora sojae in soybean and tobacco mosaic virus in tobacco cause stomatal closure, and consequently reduce transpiration of infected leaves and increase canopy temperature (Nilsson 1985b; Di Giorgio et al., 1996; Chaerle et al., 1999; McDonald and Cahill, 1999). In contrast, foliar temperature of susceptible wheat was reduced by 0.2–1.0 °C during early sporulation of Puccinia striiformis due to rust pustules rupturing the epidermis and preventing stomatal closure, followed by a significant temperature increase only in later stages of stripe rust development (Smith et al., 1986).

Transpiration is a process of diffusion with the rate of transpiration depending on the air to leaf vapour pressure deficit (ALVPD). Under humid, low-light, and high-wind speed conditions, the temperature of a dry and a wet leaf may differ by only a few degrees, whereas under high irradiance and low humidity this temperature range may exceed 15 °C (Jones, 1999b). The effect of environmental factors on the spatial heterogeneity of transpiration caused by pathogen colonization, however, has not been investigated so far.

The objectives of this study were (i) to evaluate the relationship between disease severity and MTD of cucumber leaves infected by P. cubensis; and (ii) to assess the impact of environmental conditions during measurement of MTD in order to describe the potential of MTD for the assessment and quantification of downy mildew in the field.

**Materials and methods**

**Plant material**

Seeds of cucumber (Cucumis sativus L.), cultivar ‘Vorgebirgsraupe’ susceptible to P. cubensis were germinated on moist paper at 25/20 °C for 4 d. Germinated seeds were transplanted into plastic pots (Ø 11 cm) with a 3:1 mixture of organic soil (Klasmann-Deilmann GmbH, Germany) and sand, and were grown in a greenhouse at 25/20 °C (day/night) with an RH of 70 ± 10% and a photoperiod of 16 h d⁻¹ (>300 μmol m⁻² s⁻¹, Philips SGR 140, Hamburg, Germany). Plants were watered daily with tap water and fertilized as required, and were used for the experiments when the first true leaf had fully developed.

**Pathogen**

The obligate biotrophic oomycete (kingdom chromista) P. cubensis (Berk. et Curt.) Rostovzev was maintained on the first true leaves of susceptible cucumber cv. Vorgebirgsraupe kept in the greenhouse at 25/20 °C, 70 ± 10% RH with 16 h of light per day (>300 μE m⁻² s⁻¹). Sporulation of the pathogen was induced by placing plants with the first symptoms of downy mildew in a darkened moist chamber at 20 °C and 100% RH for 18 h. Leaves bearing zoosporangia were either frozen at −20 °C for storage or used directly for inoculation.

Zoosporangia formed on the lower leaf side were dislodged with an artist’s soft brush in tap water containing 0.01% Tween-20. The first symptoms of downy mildew in a darkened moist chamber at 25/20 °C and 100% RH with 16 h of light per day (>300 μE m⁻² s⁻¹) were recorded for 18 h. The measurement chamber was maintained at 25 °C (day/night) with an RH of 70 ± 10% and a photoperiod of 16 h d⁻¹ (>300 μmol m⁻² s⁻¹, Philips SGR 140, Hamburg, Germany). Plants were watered daily with tap water and fertilized as required, and were used for the experiments when the first true leaf had fully developed.

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Thermal imaging of cucumber leaves affected by downy mildew

Disease severity desired, using a Fuchs–Rosenthal haemacytometer. About 5 ml of zoosporangia suspension of \textit{P. cubensis} was spray-inoculated onto the lower surface of the first true leaves of cucumber plants using a hand sprayer. Immediately after inoculation, plants were placed into a moist chamber at 25 °C under natural light conditions for 6 h in order to provide optimum infection conditions. For control, non-inoculated plants of the same age sprayed with water containing 0.01% Tween-20 were kept under the same conditions. Subsequently, inoculated plants and control plants were kept in the greenhouse at 25/20 °C, 70±10% RH, and a photoperiod of 16 h (>300 μE m$^{-2}$ s$^{-1}$).

\textbf{Disease assessment}

Plants inoculated with \textit{P. cubensis} were assessed daily for downy mildew development. At the first visible symptom, disease severity was assessed by visual rating of the percentage of leaf area showing characteristic symptoms of downy mildew. The necrotic area as well as the yellowish halo and the faded area surrounding the lesions were all included in the assessment of disease severity. Plants were scored visually for pathogen presence on a scale of 0, 1, 3, 5, 10, 20, . . . 90 and 100% of leaf area covered with symptoms of downy mildew using a standard area diagram according to Gaunt (1987).

\textbf{Thermographic measurements, data acquisition, and analysis}

Plants were equilibrated in the laboratory for 1 h before thermal images were recorded between 9 and 12 a.m. In general, air temperature in the laboratory was 23±1 °C, RH varied between 45% and 65%, and photosynthetic active radiation was 250±50 μE m$^{-2}$ s$^{-1}$. Variation was compensated by measuring the different treatments in an alternating sequence. For measurements under various environmental conditions, air temperature and RH in the room were set to 16, 21, or 26 °C, and at 60, 80, or 90% RH.

Digital thermal images were obtained using a VARIOSCAN 3201 ST (Jenoptic Laser, Jena, Germany) sterling-cooled infrared scanning camera with a spectral sensitivity from 8 to 12 μm and a geometric resolution of 1.5 mrad (240×360 pixels focal plane array and a 30°×20° field of view lens with a minimum focus distance of ~0.2 m). Thermal resolution is 0.03 K, and accuracy of absolute temperature measurement less than ±2 K. Digital thermograms were analysed with the software package IRBIS Plus V 2.2 (Infratec, Dresden, Germany) which allowed for correction of object emissivity after images had been recorded. However, leaf emissivity was set to 1 since relative differences in leaf temperature resulting from pathogen development were the main factors of interest of these experiments.

Colour reflectance images were taken with a digital camera (JD 4100 Z3, Jenoptic, Jena, Germany).

The transpiration rate ($E$, mmol H$_2$O m$^{-2}$ s$^{-1}$) and assimilation rate ($A$, μmol CO$_2$ m$^{-2}$ s$^{-1}$) of inoculated and non-inoculated tissue were measured for three areas (2.5 cm$^2$) with a portable porometer type CIRAS-1 with automated gas mixing and a Parkinson leaf chamber type PLC-B (PP Systems, Hitchin, UK). Flow rates were kept at 290 ml min$^{-1}$ and CO$_2$ concentration was adjusted to 480 ppm. The average leaf temperature was calculated for the three areas representing the measuring areas for the transpiration rate on healthy and infected leaf tissue. Thermographic as well as gas exchange measurements were done on one leaf per plant using six replicates per treatment.

The maximum temperature difference within healthy and infected leaves was studied by taking thermal and colour reflectance images from control leaves and inoculated leaves and recording disease severity day by day for up to 8 d after inoculation. Using IRBIS Plus V 2.2, a polygon was placed on the area representing a leaf by redrawing the cucumber leaf’s outline omitting mixed pixels on the leaf edge. For every polygon, the maximum temperature difference [K] was automatically recorded as the difference between the highest and lowest temperature within the polygon. Subsequently the software produced histograms of leaf temperature from all pixels of the marked area.

\textbf{Stomatal aperture}

The abaxial side of cucumber leaves was coated with transparent nail polish and peeled off with transparent Scotch tape. Epidermal imprints were placed on microscope slides and analysed with a Leitz DMRB photomicroscope (Leica, Wetzlar, Germany). The width of stomatal aperture was measured for 30 stomata each from non-inoculated and inoculated leaves using the software Diskus 4.2 (Hilgers, Königswinter, Germany) and averaged over three replicates.

\textbf{Membrane injury}

Leakage of electrolytes of \textit{P. cubensis} inoculated leaf tissue was investigated 5 d post-inoculation. Three types of leaf tissue were considered using chlorotic tissue, tissue surrounding chloroses, and non-inoculated leaf tissue. Leakage was assessed using a modified method described by Prohens \textit{et al}. (2004). For the cucumber leaves, eight discs (∅ 22 mm) were punched out of one leaf and washed three times with distilled water in order to eliminate electrolytes from truncated cells. Leaf discs were incubated in 100 ml of 0.4 M mannitol for 3 h. Subsequently, electric conductivity (μS cm$^{-1}$) was measured with a microprocessor conductivity meter (LF 539, WTW, Weilheim, Germany) equipped with a standard cell (Tetra Con 96, WTW, Weilheim, Germany); 0.4 M mannitol was used for reference. After the first measurement, leaf samples were autoclaved at 121 °C for 20 min to destroy the tissue completely and measured again after cooling down to 25 °C. The ratio between the first and second measurement characterizes the percentage electrolyte leakage.

According to Premachandra and Shimada (1987), the degree of membrane injury was calculated using the formula

\[ MI = \left[ 1 - \left( D_1 / D_2 \right) \right] / \left( H_1 / H_2 \right) \times 100 \]

where $MI$ is membrane injury (%); $D_1$, electrolyte leakage of diseased tissue prior to autoclaving; $D_2$, electrolyte leakage of diseased tissue after autoclaving; $H_1$, electrolyte leakage of non-diseased tissue prior to autoclaving; and $H_2$, electrolyte leakage of non-diseased tissue after autoclaving.

Leaf water content was determined by comparing fresh weight and dry weight of leaf discs (3.2 cm$^2$) punched out of non-diseased and diseased leaves.

\textbf{Statistical analysis}

All analyses were conducted using the Superior Performing Software System SPSS 11.0 (SPSS Inc., Chicago, IL, USA). Data were analysed by standard analysis of variance (ANOVA). For significant \textit{F}-values, mean comparisons were performed using a significance level of $P=0.05$. Data series were related to each other by the Pearson coefficient ($r$). All experiments were conducted at least twice.

\textbf{Results}

\textbf{Relationship between leaf temperature and transpiration rate}

Under controlled conditions, the transpiration rate of cucumber tissue proved to be linearly related to the leaf temperature as measured by digital infrared thermography. Measurements of non-inoculated and \textit{P. cubensis}-inoculated leaves were made on six consecutive days following inoculation. Analysis of data for healthy and
P. cubensis-infected tissue developing the typical sequence of downy mildew symptoms showed similar regression lines, with the slope for healthy leaves being a little bit steeper (Fig. 1). Since there was no statistically significant difference between the two regression lines, an overall correlation between leaf temperature and transpiration rate $y=28.21–0.90x$ was calculated which proved to be highly significant ($r=−0.84, P ≤0.01$).

Effect of downy mildew on leaf temperature
As early as 2 d after inoculation, infection with P. cubensis increased the overall temperature of cucumber leaves affected by almost 2 °C (Fig. 2). One day before the appearance of typical early disease symptoms (watersoaked flecks) at day 3, the MTD within the leaves was significantly larger than the MTD of non-inoculated plants. The MTD of these leaves remained largely constant during the experiment, while the MTD of infected leaves substantially increased with the appearance of chloroses associated with a locally decreased tissue temperature. Two and three days after inoculation, the calculated average largely resulted from the temperature of unaffected tissue; infection sites resulted in a few outliers. With the appearance of the first necroses 5 d after inoculation, the average leaf temperature was closer to the lowest temperature of chlorotic leaf tissue, while the substantial increase in temperature of dead tissue was restricted to some leaf spots. The concurrent presence of tissue with increased and decreased transpiration resulted in an MTD >3.5 K.

In Fig. 3, histograms demonstrate the distribution of leaf temperatures within a cucumber leaf as measured in thermograms taken 0, 3, 4, and 6 d after P. cubensis incubation. The variation in skewness with the time of incubation illustrates the dynamics in leaf temperature distribution during pathogenesis of P. cubensis: with the appearance of the first chloroses showing increased transpiration, the Gaussian distribution of healthy leaves changed to a left-skewed distribution, which changed again to a right-skewed distribution when the first necroses were produced. With the progression of pathogenesis, the frequency of leaf areas of the same temperature markedly decreased.

The effects of P. cubensis infection on leaf temperature representing leaf transpiration during pathogenesis—an initial decrease in temperature due to the formation of chloroses followed by an increase due to the necrotization of tissue—could also be demonstrated in the spatial distribution of leaf transpiration around infection sites. Seven days after inoculation, the necrotic host tissue in the centre of infection sites showing a tissue temperature 0.6 °C higher than non-infected leaf areas was surrounded by a distinct small ring of cool chlorotic tissue—1.2 °C cooler than the non-infected tissue at 21.66 °C—with increased transpiration (Fig. 4). Between these zones and the non-infected tissue, zones of intermediate temperatures were identified.

Effect on water content and electrolyte leakage of cucumber leaves
The water content of healthy cucumber leaf tissue largely remained constant at 18.1 ± 1 mg cm$^{-2}$ equivalent to 91% of fresh mass throughout the experiment. Inoculation with P. cubensis slightly decreased the water content 2 d after inoculation, which substantially increased when the first
symptoms of water-soaked tissue appeared on ~80% of leaf area 3 d after inoculation (Fig. 5). Due to increased transpiration of chlorotic tissue, subsequently the water content rapidly decreased and continued to decline to reach ~50% of that of healthy tissue 7 d after inoculation when 70% of leaf area had become necrotic. The damaging effect of *P. cubensis* infection on the integrity of cucumber plasmalemma was shown to be associated with the formation of chloroses. Five days after pathogen inoculation, the electrolyte leakage of chlorotic tissue reached almost three times the value of healthy leaf tissue (17% compared with 6%). Even the green symptomless tissue neighbouring chloroses showed an increased leakage of electrolytes intermediate to the chlorotic and the unaffected tissue, respectively. Using the formula given by Premachandra and Shimada (1987), membrane damage around chloroses was 7% and reached 12.5% for chloroses themselves. The dead tissue of necroses was not investigated.

Measurements of stomatal aperture during the early stages of pathogenesis indicated that the decreased water content of infected tissue 2 d after inoculation coincided with a slight increase in stomatal opening. In darkness, the aperture of stomata which had an average area of 25.5 μm² in non-inoculated leaves dramatically increased due to the development of downy mildew and reached 160% and 280%, respectively, 3 d and 6 d after inoculation (Fig. 6).
With illumination, stomatal aperture of infected leaves was significantly affected only 5 d after inoculation, and the effect was less pronounced.

**Relationship between disease severity and MTD**

Time series experiments showed an increase in MTD with time of incubation and hence the increase in downy mildew symptoms. Using cucumber leaves inoculated with four levels of zoospore inoculum, the relationship between the severity of downy mildew and the MTD within a leaf was investigated at three stages of symptom development (Fig. 7). After 6, 7, and 8 d of incubation, corresponding to leaves showing almost exclusively chloroses (6 d) and an increasing portion of necrotic lesions (days 7 and 8), there was a strong linear correlation between the percentage diseased leaf area and MTD. The slope of the regression curve increased with the portion of necrotic symptoms (compare days 6 and 8). For all times assessed, however, quadratic regression analysis resulted in a higher regression coefficient and turned out to be more suitable to describe the relationship.

**Influence of environmental conditions during measurements**

Environmental conditions during thermographic measurements substantially influenced the transpiration of cucumber tissue and thereby leaf temperature. At 21 °C, an increase of RH from 60% to 80% significantly reduced transpiration of healthy leaves and resulted in an increase of leaf temperature from 18.3 °C to 20.5 °C. Similarly, the increase of RH to 90% at 26 °C led to a reduced cooling effect of transpiration, resulting in a leaf temperature of 25.5 °C compared with 24.4 °C at 60% RH.

In contrast to the large variation in absolute values, the variation of transpiration within non-infected cucumber leaves hardly varied with environmental conditions (Table 1). The MTD of healthy leaves showed no significant differences throughout the experiment, irrespective of the measuring conditions. Three days after inoculation, infected leaves had a higher MTD, at least at 21 °C and 26 °C. With the appearance of downy mildew symptoms 4 d after inoculation—the average disease severity increased from 20% chloroses 4 days after inoculation to 40% at 5 d after inoculation and to 70% (40% necroses, 30% chloroses) at 6 d after inoculation, respectively—the MTD of infected leaves further increased during pathogenesis, especially at higher air temperature and reduced RH. Figure 8 demonstrates the effect of temperature and RH during measurement on the temperature distribution of a non-infected and infected cucumber leaves.
**Thermal imaging of cucumber leaves affected by downy mildew**

![Graph](image_url)

**Fig. 7.** Regression between downy mildew severity and maximum temperature difference of cucumber leaves 6, 7, and 8 d, respectively, after inoculation with *Pseudoperonospora cubensis* (n=8; measurements at 26±2 °C, 60±10% RH; *P* <0.05).

A *P. cubensis*-infected cucumber leaf for four different measuring conditions 5 d after inoculation; the leaves were adapted to the environmental conditions for 1 h before measurement. In the last column of Fig. 8, image parameters were set to maximize the temperature contrast within the displayed leaves. In contrast to the healthy leaf showing an MTD of 0.58±0.04 °C, the effect of environmental conditions on diseased leaves was very pronounced; at 26 °C, 60% RH, MTD was more than three times higher than at 16 °C, 80% RH. Since restrictions in the transpiration of diseased leaf areas (some necroses had developed) limited the cooling effect, the average leaf temperature of infected leaves was only 1.5 °C lower than the air temperature, compared with 2.8 °C for non-infected leaves.

The relationship between MTD and disease severity was also affected by the environmental conditions during measurement. The scattered plot in Fig. 9 demonstrates the positive correlation (*P* <0.01) between both parameters, irrespective of measuring conditions. Nevertheless, at 26 °C and 60% RH, the steep regression curve explained almost 85% of the variation in the percentage of diseased leaf area. Under measuring conditions reducing leaf transpiration (16 °C, 80% RH), the slope of the regression curve was not significantly different from zero and accounted for only 14% of the variation in disease severity.

**Discussion**

Digital infrared thermography proved to be a highly suitable tool for the spatial analysis of the effects of *P. cubensis* on transpiration of cucumber leaves under controlled conditions. After penetrating the leaf through stomata, this pathogen rapidly colonizes the mesophyll of its host cell producing intercellular hyphae and intracellular haustoria for the uptake of nutrients (Michelmore *et al.*, 1988; Zitter *et al.*, 1996). As experimental conditions were not conducive to the formation of sporangiophores through stomata which requires almost 100% RH for at least 6 h (Cohen, 1981), the oomycete was confined to the leaf mesophyll and had no direct effects on the leaf boundary layers. Consequently, the relationship between transpiration rate and leaf temperature was similar for healthy and infected leaves. For infected tissue, however, transpiration rates showed higher variation depending on disease symptoms: transpiration varied from 1 to 4 mmol m⁻² s⁻¹ for non-infected leaves, but varied from almost 0, for necrotic tissue at later stages of pathogenesis, to 5.5 mmol m⁻² s⁻¹, for chlorotic cucumber tissue, the first visible disease symptom occurring 2–3 d after inoculation.

Growth of *P. cubensis* in the mesophyll affected stomatal conductance of cucumber leaves prior to the formation of visible disease symptoms (Lindenthal *et al.*, 2005). Disease symptoms result from the physiological changes in plant metabolism brought about by the pathogen. The change in membrane semi-permeability is one of the earliest host reactions to pathogen attack (Wheeler, 1978; Novacky, 1983; Lucas, 1998). Modifications in the primary and secondary metabolism of plants are the results of this change. In early stages of pathogenesis (~2 d after inoculation), pathogen-induced membrane injury is likely to result in a local decrease of cell turgor and a slightly reduced water content of tissue associated with a transient increase in overall leaf temperature. Only recently, Scharte *et al.* (2005) demonstrated...
Table 1. *Influence of environmental conditions on maximum temperature difference (MTD) of non-inoculated and Pseudoperonospora cubensis-inoculated cucumber leaves during pathogenesis of downy mildew (n=6)*

Values within the same column with the same letter are statistically not significantly different, Tukey test, $P \leq 0.05$.

<table>
<thead>
<tr>
<th>Measuring condition</th>
<th>Inoculation</th>
<th>Days post-inoculation</th>
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<tr>
<td></td>
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<td>0</td>
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<tr>
<td>16 °C, 80% RH</td>
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<td>21 °C, 80% RH</td>
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<td>26 °C, 60% RH</td>
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<td>26 °C, 90% RH</td>
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Fig. 8. Effect of environmental conditions on temperature distribution, average leaf temperature ($\bar{\phi}$), and maximum temperature difference (MTD) of a non-inoculated and a *Pseudoperonospora cubensis*-inoculated cucumber leaf, respectively, 5 d after inoculation (the thermogram in the right row is shown with the settings to demonstrate maximum heterogeneity within the leaf).
the accumulation of KCl and K₂ malate (Outlaw, 2003; by the selective influx of potassium into the guard cells and (epidermal) cells (Eschrich, 1995). The turgor is regulated turgor difference between guard cells and neighbouring pathogenesis. The aperture of stomata is a function of the water content of tissue 3 days after inoculation. Stomatal biotrophic pathogen, induced a transient increase in the which, in combination with the sink activity of the chloroses. The loss of electrolytes from infected tissue is associated with disintegration of mesophyll cells; catabolic dynamic process of downy mildew development and was latent infected, symptomless tissue progressed in the highly compatible interaction with the oomycetous pathogen Phytophthora nicotianae. A temporary decrease in water availability due to a transient restriction of the xylem has been reported for the hypersensitive reaction of Nicotiana edwardsonii with tobacco mosaic virus (Wright et al., 2000). It is not known whether these effects also occur in the described compatible host–pathogen interactions.

Membrane injury which could already be detected in latent infected, symptomless tissue progressed in the highly dynamic process of downy mildew development and was associated with disintegration of mesophyll cells; catabolic activity in chloroplasts resulted in the appearance of chloroses. The loss of electrolytes from infected tissue is likely to increase the osmotic value of diseased leaf areas, which, in combination with the sink activity of the biotrophic pathogen, induced a transient increase in the water content of tissue 3 days after inoculation. Stomatal opening in the non-colonized epidermal layer induced a significant decrease in leaf temperature at this time of pathogenesis. The aperture of stomata is a function of the turgor difference between guard cells and neighbouring (epidermal) cells (Eschrich, 1995). The turgor is regulated by the selective influx of potassium into the guard cells and the accumulation of KCl and K₂ malate (Outlaw, 2003; Tallman, 2004). Barley infection by Rhynchosporium secalis caused stomatal opening due to damage of epidermal cells associated with a change in the turgor balance between guard cells and the other epidermal cells (Ayres and Jones, 1975). Three days after inoculation, stomatal regulation of cucumber leaves was still impaired. The excessive transpiration of colonized tissue rapidly led to a decrease in water content. This malfunction of stomata finally results in the desiccation of leaf tissue and total loss of transpiration—cell death associated with the formation of visible necroses. The first appearance of necroses was associated with a supra-optimal opening of stomata in the light. However, being a biotroph, P. cubensis may cause significant damage to its host plants already in early stages of pathogenesis (Lindenthal et al., 2005).

A sequence in thermal modifications resulting from pathogen attack—pre-symptomatic cooling followed by a temperature increase due to tissue desiccation—has also been described for sugar beet leaves infected with Cercospora beticola (Charle et al., 2004). The authors explained the initial cooling effect of infection by the production of a membrane-active toxin of the fungus. The biotrophic pathogen P. cubensis, in contrast, is not reported to produce toxins. The simultaneous occurrence of both symptoms in one leaf and the effect of infection on membrane function in symptomless tissue also indicate the activity of a mobile compound for the interaction of the oomycete and cucumber leaves.

Two to three days after inoculation, the heterogeneity of the transpiration rate within infected leaves was significantly higher than for healthy leaves. The MTD proved to be a simple but reliable parameter for this heterogeneity and may be used for the discrimination of healthy leaves or canopies and those with downy mildew (Lindenthal et al., 2005; Oerke et al., 2005). MTD of leaves increased during pathogenesis as initial symptoms show an increase in transpiration rate, while at later stages leaf tissue became necrotic associated with a transpiration approaching zero and a drastic increase of local temperature; the average leaf temperature may be largely unaffected. The simultaneous presence of chloroses and necroses in leaves results in the highest values of MTD, which slowly decreases when the leaf becomes completely necrotic.

Repeated measurements of various leaves differing in downy mildew severity showed a strong linear correlation between disease severity and MTD irrespective of the type of visible symptoms. The percentage leaf area affected, i.e. the number of both types of lesions, chloroses and necroses, was more important than the type of symptom; nevertheless, the appearance of necroses increased the slope of the regression curve. For all stages of pathogenesis, however, statistical analysis produced higher correlation coefficients for quadratic regressions, indicating that the positive correlation may be used only until 60% disease severity. Higher percentages of leaf area affected by P. cubensis did not increase the MTD but tended to decrease this indicator of heterogeneity in the spatial distribution of leaf temperature. Heterogeneity decreased as the leaves became almost completely diseased.

The effect of environmental conditions on the average leaf temperature and its relationship to air temperature have been described many times (Jones, 2004). Conditions favouring transpiration result in a strong cooling effect; under
conditions suppressing stomatal conductance, the difference between air temperature and overall leaf temperature is often minimal. In contrast to the absolute average leaf temperature, MTD of healthy cucumber leaves was largely unaffected by various environmental conditions during measurements. Diseased leaves, however, showed marked differences in the spatial heterogeneity of transpiration—indicated by MTD values—depending on the measurement conditions. MTD of diseased leaves was high under environmental conditions favouring leaf transpiration. According to Jones (1999b), the sensitivity of thermography for the detection of changes in leaf conductance increases with air to leaf vapour pressure deficit, net radiation absorbed, and wind speed. Downy mildew caused marked changes in tissue transpiration only when stomatal conductance of cucumber leaves was not restricted by a high RH or low temperature. Since the transpiration rate is related to a vapour pressure deficit, the effect of RH on transpiration was more pronounced than that of air temperature. The correlation between disease severity and MTD was also affected by environmental conditions. Both parameters were significantly correlated under the different measurement conditions, but the coefficient of correlation was markedly lower for conditions restricting leaf transpiration.

As the variability of leaf temperature between healthy tissue and disease symptoms is modified by environmental conditions, the MTD cannot be used either for the quantification of disease severity or as a reliable parameter for the discrimination between healthy and downy mildew-infected cucumber plants. Its dependence on environmental conditions makes it impossible to derive a threshold value for MTD. In this study, however, digital infrared thermography proved to be a powerful tool for the characterization of the different stages in the host–pathogen interaction as this remote sensing technique interferes as little as possible with the plant. MTD is a very sensitive parameter for spatial modifications in leaf temperature due to pathogen attack, which is generally restricted to a low number of cells in early pathogenesis. The mean leaf temperature (cf. Fig. 2), variance, as well as upper and lower percentiles of leaf temperature values (data not shown) proved to be less sensitive.

Digital infrared thermography alone seems not to be suitable for disease detection in the field, a prerequisite for a more demand-based use of fungicides or site-specific disease control (Paveley et al., 1996; West et al., 2003). This sensor has to be combined with other remote sensing methods offering additional spectral information and systems for the recognition of optical patterns in plant canopies (Chaerle et al., 2003; Omasa and Takayama, 2003; Jones, 2004). Also the use of reference areas or plants may be suitable (Jones, 2004), especially for the identification of wet and dry canopies.

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References
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