Application of Thermal Imaging Sensor to Early Detect Powdery Mildew Disease in Wheat

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Abstract: The potential of remote sensing as a tool to identify Powdery mildew disease in an early infection stage and to accurately quantify the severity of infection is crucial in plant disease assessment and management. Powdery mildew is one of the most harmful disease causing great losses in wheat yield. Remote sensing data were obtained in the thermal infrared spectral ranges. A greenhouse study was conducted to assess changes in leaf temperature of wheat plants during infection by powdery mildew to evaluate leaf reflectance measurements. Thermal images of plant disease under different environmental conditions in the field are a cutting-edge research. The variations in temperature between infected and healthy leaves of wheat and the variation between air and leaf-surface temperatures under greenhouse conditions were sensed for early detection of disease. Results revealed that infection with powdery mildew pathogen induced changes in leaf temperature from 0.37 ◦C (after one hour from the infection) to 0.78 ◦C at (21 days after infection with the pathogen) and metabolism, contributing to a distinct thermal signature characterizing the early and late phases of the infection. These changes in leaf temperature during Powdery mildew development resulted in a considerable heterogeneity in temperature distribution of infected leaves. The maximum temperature difference within a thermogram of wheat leaves allowed the discrimination between healthy and infected leaves before visible symptoms appeared.

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1. Introduction

To implement timely control strategies through plant monitoring, an accurate quantification of disease and damage caused by biotic and abiotic stressors in plants is required. Currently, visual disease and damage quantification methods are the most common (Guan and Nutter,2002; Steddom et al., 2004,) but these techniques are subject to bias and can be inaccurate(Richardson et al., 2001; Turner et al., 2004 and Bayoumi et al., 2014). Imprecise and inaccurate data may cause costly errors when management and policy decisions are based on biased damage evaluation. The future and the priorities of agriculture have been shifted from agricultural production towards environmental and ecological issues. The aim of modern agriculture is it not only to increase and optimize production but also to produce safe and healthy of high quality food. The over exploitation of resources in agriculture has led to environmental degradation such as plant diseases. In this regard, powdery mildew infections on wheat represent an important, increasingly growing problem. Wheat (*Triticum aestivum* L.) is one of the important field crops grown in Egypt and across the world.50 % of relative wheat yield losses may occur when mean temperature change is >2.3 ◦C, or when carbon dioxide concentration is <395 ppm (Wilcox and Makowski, 2014).

Spread and complexity of disease problems under climate change have led farmers to apply intensive chemical compounds, which result in increased the resistance of pests and pathogens (Coakley et al., 1999).Moreover, plant disease reduces global food production by at least 10% up to30% (Christou and Twyman, 2004; Strange and Sco, 2005). Specifically, powdery mildews are obligate parasitic fungi which infect wide range of crops (about 700 species of powdery mildews existing in about7600 plant species) including cereals, cucurbits, fruit trees and ornamental crops ((Bravo et al., 2004)) ,thereby causing significant losses of crop yields when compared to other plant diseases.

Mild weather results in increased powdery mildew growth. Spores germinate at leaf temperatures between 6° to 33°C; the optimum temperature for growth is 25°C. At 21° to 30°C, rapid germination and mycelium growth takes place. During favorable temperature periods, the time between spore germination and production of spores by the new colony takes only 5 days. High temperatures that do not harm the plant can harm the fungus; spores and mildew colonies can be killed at extended durations of temperatures above 33°C. The fungus is destroyed completely when air temperatures rise above 35°C for 12 hours or more if colonies are directly exposed to UV light

Wheat is most susceptible to powdery mildew infection during different stages of development under warm, humid environmental conditions that favor growth of fungus. Application of fungicides is still essential for disease management (Samobor et al., 2005).It is, therefore, important to accurately monitor the occurrence and severity of the disease in order to time fungicide applications. In this context, quantitative information on powdery mildew infection on Wheat is necessary. The conventional method for disease severity assessment in the field mainly relies on direct observation (Nilsson, 1995). This method is repeated ten time onsuming and in addition, it may vary considerably among assessors. Until now, rapid and comprehensive powdery mildew detection methods are not available in practice.

As an alternative method, remote sensing can be used to non-destructively assess plant diseases rapidly over a large area without physical contact with sampling units. Detection of crop stress is an important application of remote sensing (Piarulli et al., 2012).Thermal remote sensing is the branch of remote sensing that deals with the acquisition, processing and interpretation of data acquired primarily in the thermal infrared (TIR) region of the electromagnetic spectrum (Prakash,2000).Thermal remote sensing differs from optical remote sensing by measuring emitted radiations from the surface of the target object, whereas optical remote sensing measures reflected radiations of the target object under consideration(Sabins,1996).Thermal properties of plant leaves are affected by a complex heterogeneous internal structure that contains a certain amount of water per unit area. For that reason, it is possible to have research on individual plant with thermal remote sensing because of the versatility, accuracy and high resolution of the infrared thermography (Hu et al., 2011).Nevertheless, accurate thermal measurements depend on environmental conditions, which influence the thermal properties of the visualized crop. Therefore, calibration of images according to weather conditions is necessary for comparison between image data obtained during different measuring periods and growth seasons (Nilsson, 1995).Nevertheless, to our knowledge, not significant attention has been paid so far to the assessment of thermal imagery on Wheat disease. Thus, the main goal of this research is to evaluate the performance of thermal imagery to detect powdery mildew infection of Wheat. The specific objectives of this study were to: Assess the potential of thermal imagery to detect powdery mildew disease; evaluate the disease severity of wheat leaves infected by powdery mildew, and assess the impact of environmental conditions during measurement of wheat for the assessment and quantification of powdery mildew in the field.

2. Materials and Methods:

2.1. Artificial Infection of Wheat Plants in the Greenhouse.

To test the principal suitability of thermography for the recognition of plant infection, the responses of wheat plants(*Triticum aestivum*) to an infection by powdery mildew was analyzed in greenhouse experiments in 2012/2013 and 2013/2014, at Faculty of Agriculture Farm's of the Suez Canal University (SCU). A moderate susceptible wheat population to powdery mildew disease resulted from crossing between (Sids1 X Sakha 61) and was used in this study. This population was obtained from the Department of Agronomy, Suez Canal University from previous breeding program (Bayoumi et al., 2014).

The wheat plants were sown in 50 cm² diameter pots. Thirty pots were filled with soil that contains a mixture of clay: peat moss 1:1. All plants were watered and fertilized as required. Twenty days after sowing (plants had reached the secondleaf stage, 15 to 20 cm height), fifteen pots were selected for inoculation and the remained pots were used as a control. Inoculation was done on the youngest fully expanded leaves of five plants per pot.

Leaves were gently rubbed with clean moistened finger to remove waxy layer from the surface of leaves. Fungal spores were collected from infected wheat leaves, which were shaken before inoculation to remove the old spores; after that spores were removed with a fine brush and directly applied on the leaf surface of target susceptible plant leaves with a paint brush and by dusting spores from infected leaves plant according to (Lebedeva and Peusha 2006;Kathrin et al., 2011). Figure 1shows the typical symptoms of powdery mildew occurrence on wheat field-grown on spikes, stem of plants, and leaves, compare to typical healthy plants and leaves.

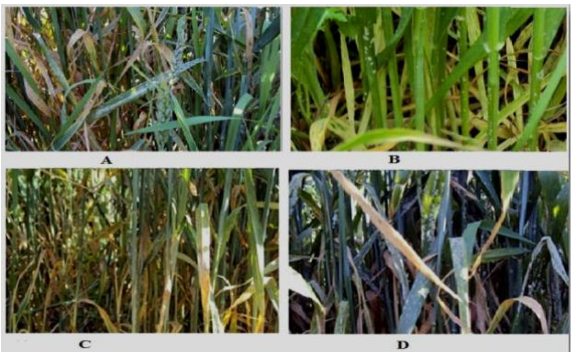

Figure1. typical symptoms of powdery mildew occurrence on wheat field-grown on (A) spikes (B) stem of plants (C) leaves and (D) typical severe symptoms on plants and leaves.

2.2. Thermo -graphic Measurements, Data Acquisition, and Analysis

The physiological changes in wheat during development of powdery mildew in leaves temperature were determined using visible and thermal imaging techniques under greenhouse by using Fluke Thermal Imager Ti32 (Fluke Thermography, USA), temperature measurement range -20 °C to +600 °C, with± 2 °C or 2 % accuracy, infrared spectral band 7.5 µm to 14 µm (long wave), field of view 23 ° x 17 ° and have infrared sensor size of 320 X 240. Both transmission correction and emissivity were 85 % and0.98, respectively. For more accuracy, the span of auto adjusted thermal image is manually set in order to detect maximum temperature of the entire display. Thermal images were taken between 12:00 AM to 3:00 PM. The temperature difference (TD) within healthy and infected leaves was studied by taking thermal and color reflectance images from control leaves and inoculated leaves and recording disease severity day by day after inoculation. Digital thermal image analyzed with the software package Smart View® version 3.5.31, which allowed for correction of object emissivity after images had been recorded. However, leaf emissivity was set to 0.98 according to (Lindenthal et al., 2005).The minimum differential between leaves in healthy plant and infected plant were determined using the following equation according to (Chiwaki et al., 2005).

Temperature Differential (T.D) = Temperature of leaves in infected plant – Temperature of leaves in healthy plant

2.3 Humidity, Air Temperatures and Leaf Area Determination

Total leaf areas of wheat and disease severity were estimated from captured images using Image J software (version 1.48s, USA). Disease severity was measured as relative between total leaf area and the infected leaf area as a percentage according to (Feng et al., 2011) and (Nunes -Maciel et al., 2013. Infrared thermal images of wheat plants were obtained from the period before the appearance of external symptoms until the disease conditions existed. Each thermal image was taken for two wheat plants healthy and infected in each frame of image. Immediately after inoculation, disease assessment inoculated plants were assessed daily for powdery mildew development and taken the image. At the first visible symptom, area measurements for disease rating used the Image J software (The lesion as well as the yellowish halo and the faded area surrounding the lesion were all included in the assessment of disease severity. Color pictures were taken with a digital camera (Canon A 2500) at the time of taken thermal image. Air temperature and Relative Humidity were recorded at image time. Humidity and air temperatures in the greenhouse were periodically measured using Humidity and Temperature Meter (Jenway, model 5075, serial No.: 43424, USA) in the same time of capturing thermal images of healthy and infected leaves.

After scanning, the leaves and shoots of the measured plants were harvested and the fresh weight was determined at once. The visible degree of infection was monitored according to the guidelines of (Chaerle et al., 2006) on a percentage scale. Plant samples were then dried at 60 ◦C and total dry matter was determined.

3. Statistical Analysis:

All analyses were conducted using SPSS 11.0 (SPSS Inc., Chicago, IL). Data were analyzed by standard analysis of variance. When the F values were significant, mean comparisons were performed with the least significant difference value at significance level of P= 0.05. All experiments were conducted at least twice.

4. Results and Discussion:

4.1. Changes in Leaf Temperature

No visible signs of infection were recognized in inoculated leaves between the beginnings of the experiment and the first measurements. During the following 20 days, number of mildew hyphae and conidia increased on the leaves and led finally to the senescence of the plants. However, even when the leaves were populated with mildew colonies in the late stage of infection, green islands of apparently healthy leaf areas could be observed among the colonies. Table 1 shows changes in temperatures

(T.D) of normal and infected leaves after infection with *Blumeria graminis tritici* under greenhouse .The relationship between the severity of powdery mildew and temperature differential ºC (T.D) within a whole leaves temperature of wheat plants were investigated at 14 periods of symptoms development to determine the changes in leaves temperature of wheat as a result infected with powdery mildew. After one hour from the inoculation, there was slightly decrease in temperature differential of the infected wheat leaves (TD= -0.02 ºC) with no symptoms were shown. After that, the decreasing in TD of infected leaves increasing with increasing of disease severity and spreading of lesion leaves reached to the highest decreasing (TD= -0.64) at 6 day after inoculation(DAI), where the disease severity (DS=2.41%). Thereafter, there is a gradually retreating in decreasing of TD at eight DAI from TD= -0.62 and DS=2.46% to TD= -0.37 and DS=30.9% at 21 DAI.

Table 1: *changes in temperatures (t.d) of normal and infected leaves after infection with Blumeria graministritici under greenhouse.*

DAI	T.D ºC	DS %	Air temperature ºC	Relative humidity %
0.04	-0.02	0	25.90	82.00
0.08	-0.05	0	25.50	82.00
2	-0.32	0	28.50	82.00
3	-0.55	0	25.50	87.00
5	-0.63	01.03	27.90	85.00
6	-0.64	02.41	25.00	79.00
8	-0.62	02.46	21.00	80.70
9	-0.61	09.09	26.50	83.00
12	-0.45	15.48	23.90	80.60
13	-0.44	19.20	25.00	81.00
14	-0.45	25.20	24.60	80.60
17	-0.42	26.30	29.90	78.50
18	-0.40	26.80	30.88	81.00
21	-0.37	30.90	30.00	85.00

DAI = days after inoculation with pathogen, Ds% = disease severity of powdery mildew %, * 0.04 DAI = 1 hour after infection with pathogen, ** 0.08 DAI = 2 hours after the infection

4.2. Visual Diagnosis of Temperature Differentials on Infrared Thermal Images

The aforementioned disease symptoms were observed at 5 DAI after the infection (DAI), Figure 2 illustrate representative pseudo color thermal images in the thermal infrared region from a healthy and infected, respectively which explains the dynamics of leaf temperature of healthy and infected leaves which inoculated with powdery mildew. The analysis of thermal images showed that powdery mildew of wheat development caused a decrease in leaf temperature of inoculated leaves at the point of effective infection and before the symptoms become visible. This might be due to dense of colonies and impact of the disease on the physiological processes in the infected leaves, such as the concentration of salicylic acid and Stomata lock-open, as well as the water potential. The results were agreement with (Stoll et al., 2008; Oerke and Steriner, 2010).

They were reported that dense colonies of powdery mildew, in contrast, were associated with a temperature only slightly lower 0.2 C than healthy leaves. In later stages, powdery mildew tends to slightly increase leaves temperature because of reduced water potential of disease leaf area and spatial and temporal analysis of leaf temperature improved the differentiation between healthy and infected leaves. Prokopov et al., 2010 reported that powdery mildew infection caused decrease in salicylic acid content after 3 days, but on the 7thday of infection it increased significantly in wheat and powdery mildew attack disrupted stomatal behavior and impair stomatal opening, and hence leaf water conductance .The present results,however indicate that the effect of infection can be detected as early as three days after inoculation, as demonstrated by changes especially in leaf temperature and before symptoms become visible.

4.3. Leaves Temperature Distribution

Table 2 shows the leave temperature distribution according to leaves position. Inoculated plant after two days of inoculation (2 DAI) showed slightly decreasing in leaf temperature in lower and middle leaves which recorded in lower leaves (TD =-0.61) and middle(TD =-0.50), while TD increasing (TD= 0.15) on the upper leaf in infected plant. After 3 days of inoculated showed slightly decreased in TD on upper (TD=0.12), while middle and lower leaves still decreasing (TD=-0.86, and 0.91respectively). In all of previous days, no symptoms appearance in infected leaves plant. At 5day after inoculation, symptoms began to appearance on the lower leaves as small lesion, where TD of lower and middle leaves continued decreased (ΔTD=-0.99, and-0.98respectively).

Upper leaf is still decreasing (TD=0.11). The 6 and 8 days of inoculation were showed the maximum decreasing in lower leaves (ΔTD=-1.04, and -1.07respectively). Middle leaves show stability (ΔTD=-0.-99) and upper leaves (TD=0.10, 0.11respectively). The 9 DAI showed increase on TD on lower leaves (-0.97), while stability TD in middle and upper leaves (TD=-0.98, 0.10respectively). The

12 DAI showed decrease on TD of upper and middle leaf temperature suddenly (TD = -0.32, -1.08respectively), and increase TD on lower (TD=0.06). On the other hand, from 13 DAI to 21 DAI were showed continuation increasing but slightly on TD on lower leaves while a decreased in TD upper leaves and beginning increasing in bottom leaves.

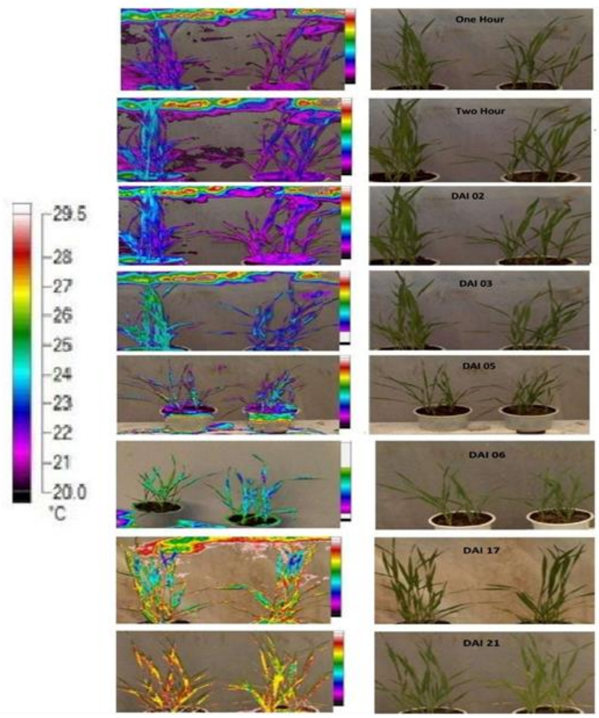

Figure 2. Dynamics of leaves temperature of healthy wheat leaves (right) and thermal images of leaves inoculated (left) with *Blumeria graminis tritici* after one hour to 21days after inoculation

Table 2: *Distribution of leaves temperature according to position of leaves plants.*

DAI	Temperature differential (ºC) T.D on upper leaves	T.D on Middle leaves	T.D on Lower leaves	Mean TD plant ºC
2	0.15	-0.50	-0.61	-0.32
3	0.12	-0.86	-0.91	-0.55
5	0.11	-0.98	-0.99	-0.62
6	0.10	-0.99	-1.04	-0.64
8	0.11	-0.99	-1.07	-0.65
9	0.10	-0.98	-0.97	-0.62
12	-0.32	-1.08	0.06	-0.45
13	-0.41	-1.00	0.09	-0.44
14	-0.50	-0.97	0.13	-0.45
17	-0.79	-0.72	0.26	-0.42
18	-0.90	-0.69	0.40	-0.40
21	-1.70	0.15	0.45	-0.37

4.3. Visualization of Leaves Temperature with Plant Position

Figure 3 shows the distribution of leaves temperature according to position on leaves plant using thermal image techniques. A decreasing in the leaves temperature of the lower and middle leaves compare to upper leaves in plant and before symptoms become visible. Thereafter at five DAI symptoms were appeared on the lower leaves first, coincided decrease in leaves temperature. Decreasing in leaves temperature coincided with the progression of the disease on the leaves of the plant with the occurrence of stability in leaves temperature of middle leaves this decreasing reach to a maximum at 8 DAI day where (TD = -1.07). Leaves temperature of lower leaves were increasing at 9 DAI (TD=-0.97) to reach its maximum increasing at 21 DAI where (TD =0.45) as a result of yellowing leaves and death tissue and destruction of the cells as a result of developing the disease. However, middle leaves were decreased in leaves temperature at 2DAI (TD=-0.5) and reach to a maximum decreasing at 12

DAI (TD = -1.08) coincided with a slightly decreased in the leaves temperature of upper leaves (TD=-0.32). The temperature of middle leaves increasing at 13 DAI (TD=-1) because of the beginning of the yellowing of leaves and death of some of the tissue. Develop of the disease reached to maximum increasing at 21 DAI (TD =0.15) as a result an increased rate of death and death tissue and destruction of the cells while continuing decreasing in the upper leaves temperature.

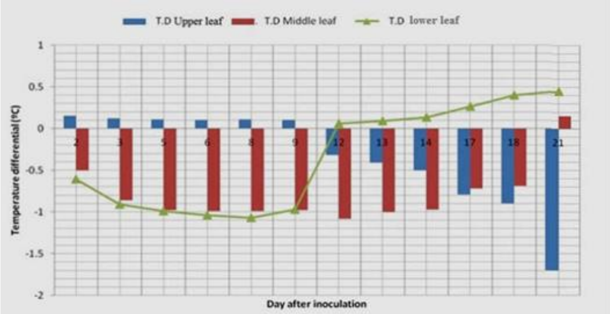

Figure 3. Distribution of leaves temperature according to position on leaves plant using thermal image techniques

5. Overall Discussion:

Crop protection decisions are currently determined at the greenhouse scale. As demonstrated by Secher (1997), disease control might be optimized if fungicides are applied on a site- specific scale, resulting in more sustainable agricultural cropping systems and reduced environmental impact. In order to apply fungicides on a site-specific scale, non-intrusive sensor technologies for disease identification and tools to support fungicide spray decisions must be developed. Remote sensing and reflectance measurements have gone through rapid development over the past two decades and there is a trend towards the use of images in the application of stress identification for precision farming (Deguise and McNairn, 2000). The duration of the latency period is particularly important for powdery mildew, whose infection does not strictly depend on particular environmental conditions, like rainfall of the presence of free water on plant organs. Infection by this pathogen is virtually continuous and therefore, the epidemic progression mostly depends on the daily dose of inoculums able to infect the host, which in turn depends on how many infection cycles have been completed, leading to sporulating powdery mildew colonies (Xu, 1999). Based on our findings, how the powdery mildew of wheat develop on leaves and the possibility of followed by using of thermal imaging as a new technology to predict the powdery mildew disease at three days before symptoms become visible. Reliable differentiation between normal and infected leaves was occurred after 1 hour from the infection due to the formation of fungal conidia under epidermal layer. After hours from infection, the germ tube adsorbs water and solutes from host cell wall to facilitate the infection process (Edwards, 2002).Powdery mildew consumes nutrients from the infected plant by their haustoria which grow inside epidermal cells (Bravo et al., 2004) and therefore within 2-4 days micro-colonies are visible on the leaf surface as gray fungal growths on the lower leaf (Figure 2 and Table 2).Powdery mildew infection causes changes in plant carbohydrate metabolism, as well as salicylic acid and oxylipins involved in inducing defense (Bockus et al., 2010). The reaction between pathogen and wheat leaves was occurred after 7 days from the artificial infection of powdery mildew pathogen, leading to the appearance of fungal growth as a disease symptom on leaves.

The results were agreement with those of (Awad et al., 2014 and Bayoumi et al., 2014) They were reported that thermal infrared has been proved by various researchers to be a useful tool for the pre-symptomatic effect of disease and pathogen on plant and allowed to tracking of disease progression even at the early pre-symptomatic stage. Xin, et al.,2012 reported that during plant-pathogen infection, the physiological state of the infected tissue is altered, such as changes in photosynthesis, transpiration, stomata conductance, accumulation of salicylic acid and even cell death this were related to leaves temperature. Ullah, (2013) was reported that foliar disease can directly be detected by using thermal images which were able to identify powdery mildew of barley and wheat and have the potential to identify and quantify with high spatial resolution management zones in disease control and associated pathogens, as they are sensitive to physiological disorders associated with fungal attack as well as disease (Awad et al., 2014). In addition, leaf diseases often affect plant transpiration. A sequence in thermal modifications resulting from pathogen attack pre-symptomatic cooling followed by a temperature increase due to tissue desiccation described for sugar beet leaves infected with *Cercospora beticola* (Chaerle et al., 2006).A proven results, which can take a particular decision enables us to reduce appearances of powdery mildew of wheat, spread early, and thereby reduce losses in crop of wheat.

6. Conclusion:

Thermal imaging has been growing fast and playing an important role in plants disease detection. In this study, a disease identification system was developed for Wheat powdery mildew infections. To improve the system for identifying powdery mildew infections, a thermal imagery was utilized, and results showed that the ability of this system was acceptable. In future research, it is desirable to develop intelligent platform for real-time diseases identification. This could be achieved by multi-sensing systems equipped with an artificial light source and a multi-sensor platform that moves in the crop fields and detects fungal diseases on plants' leaves. In order to increase the ability of real-time detection of wide range of fungal diseases, our ongoing research is currently focused on developing early warning system for disease detection.

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