

# Plant Stress Detection by Thermography

Subjects: Plant Sciences

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Leaf and canopy temperature is a valuable indicator of the physiological status of plants, responding to both biotic and abiotic stressors. Thermography, often combined with other imaging sensors and data-mining techniques, is crucial in the implementation of a more automatized, precise and sustainable agriculture.

Keywords: Remote sensing ; proximal sensing ; biotic stress ; plant stress

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## 1. Introduction

Environmental conditions driven by climate change and infections are great challenges that need to be overcome by modern agriculture. The economic loss in agriculture caused by increasing environmental pressures could reach an annual 0.3% to 0.8% of projected global gross domestic product by the end of the century <sup>[1]</sup>. Moreover, plant diseases are the main cause of the drop in production and economic losses in agriculture worldwide, reaching yield losses of 20 to 30% depending on the crop <sup>[2]</sup>. Current practices and social activities -such as intensified monoculture in large areas, the use of genetically uniform plant varieties and the development of global supply chains and logistic activities- contribute largely to the widespread of plant disease epidemics and rapid pathogen evolution <sup>[3]</sup>.

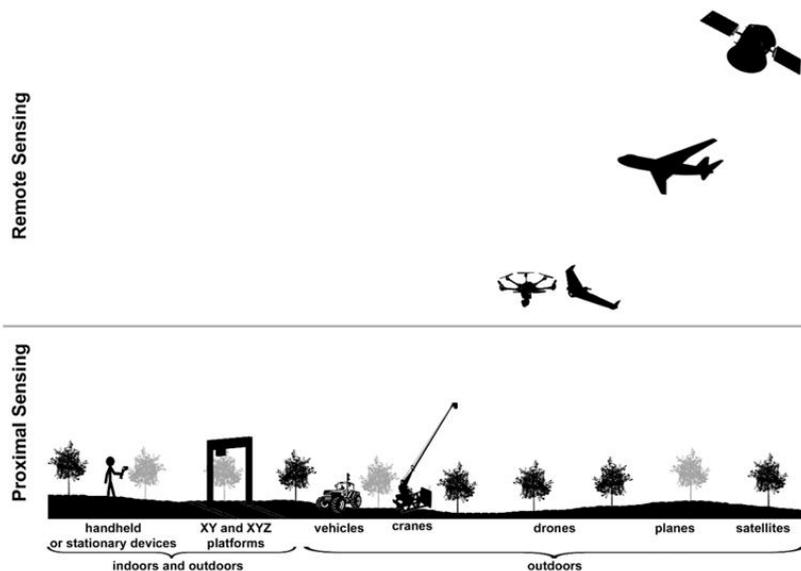
Plant phenotyping based on imaging techniques is a pertinent approach to quantify the appearance and performance of crops under different environmental conditions while addressing the spatial heterogeneity of crop fields. Therefore, plant phenotyping, applied to precision agriculture, is a valuable tool for the diagnosis and detection of plant stress, even in the absence of symptoms. Optical sensors have been used to study a) the response of plants to pathogens, pests and abiotic stressors; b) to identify primary disease foci; c) to monitor resistance or susceptibility of different plant genotypes to specific stress factors; d) to evaluate the severity of symptoms; e) and to assess plant biomass and yield <sup>[4]</sup>.

Stomatal activity is one of the most important physiological traits for plant growth and development. It plays a crucial role in the carbon and water balance by controlling photosynthesis and transpiration <sup>[5]</sup>. Hence, stomatal conductance to water (gs) is related to yield and to the tolerance of environmental stresses <sup>[6]</sup> and correlates strongly with leaf temperature <sup>[7][8][9]</sup>. Thermal long-wave infrared (TIR) cameras (or simply thermal cameras) are calibrated sensors able to record emitted radiation in the thermal range (8–14  $\mu\text{m}$ ) and provide images representing temperature values per pixel. Thus, conventional, time-consuming ground-based gs measurements can be feasibly replaced by thermal images evaluating plant physiological status at different scales in short periods of time <sup>[10][11]</sup>. Additionally, highly sensitive thermal cameras with a relatively simple operational procedure have become more available to research groups, at a lower cost and at higher spatial resolution <sup>[12][13]</sup>. Moreover, TIR imaging is a very valuable method of stress detection prior to the appearance of symptoms since it operates out of the visual range of the spectrum <sup>[14]</sup>. However, some considerations must be taken when interpreting thermograms in terms of gs since plant surface temperature is mainly driven by environmental factors such as air temperature and relative humidity.

Despite the wide use of thermography applied to agronomy, this technique per se has a very limited capacity for diagnosis. Indeed, temperature raises may be due to stomatal closure, which is on its own an unspecific mechanism of plant defense against both abiotic and biotic stressors. Even more, temperature raises can also respond to a decreased capacity for water evaporation or even to the loss of vegetation <sup>[10]</sup>. In contrast, some stresses can cause temporary decreases in leaf temperature. That would be the case of some pathogens that can interfere in the regulation of stomatal movements to favor their entry to the mesophyll <sup>[15]</sup>. As a consequence, alterations in TIR parameters stand as ambiguous clues for diagnosis. Moreover, abiotic stress factors (mostly drought, soil salinity or extreme ambient temperature) also cause increases in canopy temperature. This is most important under natural conditions, where abiotic stressors are difficult to assess and avoid. Keeping in mind that under such conditions, several stress factors often affect the vegetation simultaneously, possible misinterpretations of TIR data would lead to inaccurate determination of the incidence and severity of a particular pathogen infection or even to a wrong diagnosis.

To overcome the restraints of thermography as a diagnostic technique, authors have increasingly applied it in combination with other imaging techniques. Roitsch et al. [16] and Sperschneider [17] reviewed the implementation of TIR cameras with other sensors, such as RGB, multi- or hyperspectral cameras, in phenotyping platforms. The identification of spatial and temporal patterns of TIR parameters in combination with other relevant vegetation indices (VIs) could be of great help to establish robust methods for the early diagnosis in crop fields. However, a preliminary analysis of a given plant-stressor interaction is desirable to obtain a stress-specific signature [18].

The use of complementary imaging techniques provides valuable and very complex information. This complexity is caused by the increasing number of dimensions that can be considered and the incessant improvements in their spatial and temporal resolution. To enhance our capacity for data analysis, most recent works have included data-mining in their analysis. This multidisciplinary approach, based on probability theory, statistics, decision theory, visualization and optimization, outperforms more conventional statistical analysis in terms of finding patterns in data [19][20]. Classifiers are algorithms able to learn patterns from a database of known samples and, based on that knowledge, to identify or categorize new samples [21]. When applied to agriculture, this approach facilitates the interpretation of data and the decision-making process to such an extent that no study appears to be complete without this type of analysis.



**Figure 1.** The most common platforms implementing thermal cameras at proximal and remote sensing scale.

Thermography is extensively used for stress detection from lab to field scale by either proximal or remote sensing (Figure 1). On one hand, proximal sensing (mainly in growth chambers and greenhouses) refers to imaging single leaves or entire plants from a close distance to the target. The camera could be mounted on a static stand or small and medium-size robots, including high-throughput platforms. On the other hand, remote sensing refers to measurements taken in open fields with imaging sensors implemented on a wide range of devices, such as cranes, vehicles, robots and unmanned aerial vehicles (UAVs; in which the image resolution depends on the flight height) to cover whole crop fields. Even larger areas (district to region scales) can be tackled thanks to the sensors onboard satellites (i. e. ASTER, Sentinel-3, ECOSTREES, or Landsat-7 and 8, among others). These sensors differ in their spatial resolution and also on the time resolution, depending on the revisiting frequency of the satellite over the particular area.

Physical and technical aspects of thermography, such as scientific principles applied to measurements and data correction methods [22][23][24][25], as well as practical considerations affecting thermal imagery for plant phenotyping [26][27], have been recently reviewed. Other aspects related to thermography, such as available imaging sensors and evaluation of their potential [28][29], their use in phenotyping platforms [16][30][31] or UAVs [24][32][33][34], and their applications have also deserved special attention by many authors. Moreover, other TIR applications have also been thoroughly reviewed elsewhere [18][22]. Furthermore, very recent works reviewed the state of the art in the application of artificial intelligence to stress detection [17][20]. Despite the vast number of works addressing the use of thermography for plant stress detection, little attention has been paid to the physiological perspective of temperature alterations related to stressed plants. Attending to this need, Pineda et al [35] revised the most relevant applications of thermography to agriculture with the main aim of providing TIR camera users with a collection of host plant–pathogen systems described in the literature in terms of thermal response and the mechanisms of plant defense involved.

## 2. Thermography Applied to Biotic Stress Detection at Proximal Sensing Scale

Stomata are the main natural entry of pathogens into the plant tissues. Hence, the regulation of the stomatal movements plays a key role in plant defense against microbes [36]. The recognition of a potential pathogen by plants usually triggers stomatal closure; to counteract this defense response, some pathogens are able to override the plant signaling pathways to activate stomatal aperture [37]. Other effects of pathogen infection include disturbances on leaf development, cell wall and leaf cuticle composition or integrity, alterations in the plant metabolism or even necrosis of the tissues. These physical and chemical disturbances affect the plant water status, which can be monitored by thermography [38][39][40].

### 2.1. Viral Infections

Even when some virosis cause alterations neither in  $g_s$  nor in leaf temperature [41], thermography is very often helpful in tracking viral infections on plants. Most of those works report temperature increases between +0.5 and +1.5 K; on the contrary, some works reported decreases in leaf temperature of -0.5 K relative to mock-controls. Hypersensitive response (HR) to Tobacco mosaic virus (TMV) could be visualized as spots of elevated temperature before any disease symptoms became visible on tobacco leaves. Those spots were confined to the site of infection as a result of the HR triggered by salicylic acid. On the other hand, no thermal response was observed on susceptible tobacco-TMV-infected plants [42]. In the case of Pepper middle mottle virus (PMMoV)-*Nicotiana benthamiana* plants, virus spreading through asymptomatic leaves could be first detected by an increase in temperature on the tissues around the main veins. This effect extended to adjacent tissues before the spreading of the virus across the midrib of the leaf, as shown by immunolocalization. Interestingly, the thermal symptoms caused by the most severe strain of PMMoV were detected before those caused by the less virulent one [43]. Among other tobamoviruses, Cucumber mosaic virus (CMV) and Cucumber green mottle mosaic virus (CGMMV) have a particular impact on cucumber production. Plants infected with CMV displayed homogeneously higher temperature in the whole inoculated leaf relative to the controls, whereas leaves of CGMMV-infected cucumber plants showed a heterogeneous temperature pattern, consisting of cold spots at the infection sites. However, none of those changes was detectable presymptomatically. Nevertheless, classificatory algorithms could early detect the infection caused by each pathogen when combining data obtained by TIR, fluorescence and hyperspectral imaging [44]. Another virus of agricultural interest is the Sweet potato feathery mottle virus, which is the most widespread virus that infects sweet potato plants, causing devastating problems when co-infecting with Sweet potato chlorotic stunt virus. Higher leaf temperatures were associated with more severe symptoms; thus, plants co-infected with both viruses displayed higher temperatures than single virus-infected plants [45].

### 2.2. Bacterial Infections

In the case of bacterial-infected plants, literature reports temperature changes relative to the controls ranging between -3.3 K and +3 K. The tumor caused by *Agrobacterium tumefaciens* in the hypocotyl of castor oil plant produces irreversible disruption of the plant epidermis and hence, the tumor lacks a protective cuticle against water loss. Moreover, stomata located at the tumor edge are hypertrophied and non-functional; as a consequence, the tumor surface was the coldest part of the plant [46]. Some works have studied the role of plant defense elicitors produced by bacterial pathogens. That is the case of the proteinaceous harpin synthesized by the bacterium *Erwinia amylovora*. When infiltrated in the leaves of *Nicotiana glauca* wild plants, harpin induced a marked presymptomatic cooling at the inoculation sites, followed by an increase of temperature during the HR [47]. The same trend was described for spots of bacterial canker caused by *Pseudomonas syringae* pv. *actinidiae* on kiwifruit leaves [48]. The decrease in temperature localized at the infection sites could be related to bacterial virulence factors that actively open the stomata by interfering with hormonal signaling pathways leading to stomatal closure [49]. On the other hand, it is well known that recognition of some pathogen-associated molecular patterns triggers stomatal closure to impede bacterial entrance through these natural apertures [15]. In bean plants, *P. syringae* pv. *tomato* DC3000 induces an HR, whereas *P. syringae* pv. *phaseolicola* 1448A produces systemic infection. First symptoms are evident after 10 h and 2 days post-infection, respectively. Thermography could presymptomatically reveal those bacterial challenges as an increase of temperature of the inoculated areas at 1 and 2 h post-infection in the case of HR and systemic infection, respectively [50].

Several works have addressed the effect of the *Dickeya dadantii* infection on plant transpiration by thermography. This bacterium usually produces soft-rot, a characteristic tissue maceration, as in the case of *N. benthamiana* infected at high inoculum dose. After mechanical infiltration, the temperature of the affected areas raised presymptomatically, linked to an increase in hormones controlling stomatal closure. In subsequent days, the temperature continued rising, affecting the whole leaf. However, *N. benthamiana* can activate an efficient defense response against *D. dadantii* when the plant is inoculated at doses resembling the natural infection. In this case, after an initial increase in temperature of the infiltrated area, infected plants could recover, and temperature subsequently decreased [51]. Independently of the inoculum dose, *D.*

*D. dadantii* does not cause tissue maceration on melon leaves. It produces brownish spots at the inoculation sites that evolve chlorosis in the surrounding tissues in successive days; those symptoms appeared earlier when high dose concentrations were applied. Only infiltrated areas shown increased temperatures at the beginning of the infection process when inoculated at a low dose; the whole leaf finally displaying higher temperature respecting to the controls. However, when a high inoculum dose was applied, the whole melon leaf displayed a higher temperature [52]. The whole leaf of another cucurbit, such as zucchini, displayed higher temperature relative to the controls when inoculated with *D. dadantii*, whereas visual symptoms (different degrees of chlorosis proportional to the inoculated dose) were circumscribed to the infiltrated spots [53][54]. Features extracted from TIR and multicolor fluorescence images were used to feed algorithms, which provided a good performance of classification of plants into categories of infected and controls in both melon and zucchini plants [52][53].

### 2.3. Interactions with Pathogenic Fungi and Oomycetes

Thermography has been widely used to assess the effect of fungi and oomycetes on host plant transpiration. Depending on how these pathogens interact with host plants, initial phases of pathogenesis can induce increases or decreases in leaf temperatures affecting only the inoculation sites or whole leaves. Subsequent symptoms development may cause transitory drops in temperature as the affected cells die and lose water. Later infection phases usually lead to an increase in temperature due to the lack of natural cooling of the necrotic tissues. Concerning healthy areas, it is possible to find in the cited literature that biotrophic fungi and oomycetes can cause temperature decreases ranging from  $-0.1$  to  $-2.5$  K, whereas increases have been registered from  $+0.6$  to  $+2$  K. For their part, hemibiotrophic pathogens can decrease leaf temperature between  $-0.6$  and  $-2.2$  K, or contrary, increase it in  $+0.4$  to  $+7.5$  K. Finally, it is possible to register temperatures decreases between  $-2$  and  $-5$  K and increases ranging from  $+0.3$  to  $+9$  K when necrotrophic fungi are studied by TIR imaging.

Concerning infections caused by biotrophic fungi leaves from wheat plants infected with *Blumeria graminis* or *Puccinia striiformis* (fungi causing powdery mildew and stripe rust, respectively) displayed low temperatures due to the very low resistance to water evaporation of growing mycelia [55][56]. The phytopathogens *Pseudoperonospora cubensis* and *Podosphaera xanthii* are the causal agents of cucumber downy mildew and cucurbits powdery mildew, respectively. In cucumber leaves, these pathogens produce infective spots with lower temperatures than the surrounding healthy areas due to an abnormal stomatal opening. In the case of *P. xanthii* infection, the thermographic detection was not presymptomatic, whereas disease caused by *P. cubensis* could be revealed one day before the appearance of symptoms. Under laboratory conditions, the MTD increased during both pathogenesis and was related to disease severity [44][57][59][58]. Despite being a biotrophic oomycete, *P. cubensis* also produces necrosis in the latest infection phase associated with an increase in cucumber leaf temperature [57][59]. Leaves of rose infected with the fungus *Podosphaera pannosa* var. *rosae* (causal agent of powdery mildew in roses) showed a presymptomatic decrease in temperature. Furthermore, two algorithms were trained on features extracted from TIR images, obtaining high accuracy in classifying healthy and infected plants [60]. Raza et al. [61] also used information extracted from TIR and RGB pictures to automatically detect tomato plants infected with *Oidium neolycopersici* in a presymptomatic way, but the work did not address the physiological changes caused by the fungus.

Regarding hemibiotrophic fungi, TIR imaging could describe the severity of the infection, which could be related to the disease stages but also to the host plant resistance to the pathogen. Thus, Oerke et al. [62] and Belin et al. [63] analyzed apple trees suffering from apple scab. In that case, the detection of infection, as well as differences in the virulence of several *Venturia inaequalis* isolates infecting apple trees, were detected more accurately by thermography than by chlorophyll fluorescence imaging. The thermal response was presymptomatic and consisted of spots of decreased temperature due to the subcuticular growth of *V. inaequalis*. A soilborne fungus, *Rhizoctonia solani*, induces necrosis in lettuce plants, which could be visualized as an increase in leaf temperature and MTD [64]. The damage produced by another soilborne fungus, *Fusarium oxysporum*, to whole cucumber leaves could be detected as a presymptomatic raise in temperature induced by abscisic acid, followed by a slight decrease as wilt symptoms developed. Finally, the temperature of whole leaves presenting necrosis increased again [65]. On the contrary, pea plants infected with this fungus showed an early and slight reduction of temperature compared to the control plants. At an advanced stage of the infection, the leaf temperature increased above control levels in the case of susceptible plants, whereas the temperature of those showing *F. oxysporum*-resistance remained similar to the controls [66].

In the case of infections caused by necrotrophic fungi, sugar beet plants inoculated with *Cercospora beticola* (causal agent of *Cercospora* leaf spot) displayed spots of presymptomatic low temperature corresponding to the infection sites. Cold spots appeared progressively until covering the whole leaf surface, whereas lesions were hardly visible by the naked eye. Toxins influencing cell membrane permeability and produced by *C. beticola* could account for this temperature decrease [42]. *Aspergillus carbonarius* is a fungus causing sour rot of grape berries and produces ochratoxin-As, toxic for

humans. Mycelium growth areas showed low temperatures that identified affected fruit sites at the very early stages of *A. carbonarius* infection [67]. Thermography also revealed that *Alternaria alternata*, *A. brassicae* and *A. brassicicola* caused a decrease of temperature during the first seven days of infection on oilseed rape leaves, followed by a temperature increase in successive days. However, those plants inoculated with *A. dauci* only developed increases of mean temperature at 21 days after inoculation [68]. The infection with *Botrytis cinerea* (gray mold) on bean plants caused a presymptomatic increase of temperature at the infection sites. In subsequent days, leaves developed hot brownish necrotic lesions surrounded by a lower temperature area [69]. On the contrary, *B. cinerea*-infected roses first showed a decrease of temperature at regions where lesions were initially formed, followed by a rise in temperature once necrosis occurred [60]. *Rosellinia necatrix* (soilborne fungus causing white root rot) is one of the most important constraints to production for a wide range of woody crops such as avocado. In the late phases of *R. necatrix* infection, trees undergo a water deficit in the aerial part due to failure of the root system [70]. As a result, infected plants experienced a significant increase in leaf temperature from the early symptomatic stage onwards [71]. Furthermore, infected avocado trees in an orchard could be detected by thermography (see Section 3) [72].

## 2.4. Herbivory and Parasitic Plants

Herbivore insects disrupt the integrity of leaves, leading to uncontrolled water loss from wounds and triggering intricate processes that affect gas exchange also in the remaining leaf tissue. Thus, it is possible to find temperature drops from  $-0.3$  to  $-3$  K in the literature. TIR images of soybean leaflets affected by corn earworm caterpillars (*Helicoverpa zea*) showed that leaf areas adjacent to wounds were cooler than distant regions or sister leaflets [73]. The same leaf temperature pattern was visible after injuries caused by cabbage looper (*Trichoplusia ni*) instars on *Arabidopsis* [74], tobacco hornworm (*Manduca sexta*) on *Nicotiana attenuata* [75], or larval gypsy moth (*Lymantria dispar*) and gall damage produced by midge flies (*Harmandia* sp.) on aspen leaves [76]. When elevated CO<sub>2</sub> was applied to the atmosphere, the cooling effect of gall formation on remaining leaf tissue was reduced [76].

Thermography was also useful in the detection of plants infested by parasites, revealing temperature increases (from  $+0.4$  to  $+0.9$  K) due to a reduction in the plant water uptake by affected roots. Examples of these interactions are the nematode *Heterodera schachtii* with susceptible sugar beet cultivars [77], as well as the obligatory root parasitic plant *Orobanche cumana* (broomrape) and sunflower [78]. The early detection of broomrape by nondestructive techniques was unprecedented since natural infestation causes subtle alterations on host physiology and proceeds unnoticed until the emergence of the floral shoots, by the time of sunflower bloom.

## 3. Thermography Applied to Biotic Stress Detection at Remote Sensing Scale

In crop fields, thermography has facilitated the detailed analysis of crop fields affected by pathogens, helping in the localization of areas where plants are affected and requiring urgent intervention [79]. In general, pathogen infected plants show changes their temperature of  $-2$  K to  $+3.1$  K, relative to the temperature of healthy plants; however, it is worth noticing that these values should be handled with care depending on the sensitivity and the accuracy of the used TIR cameras. The identification of potentially infected plants using classifiers provides accuracies ranging between 59-89%, depending on the applied algorithm and parameters used to feed it.

In a susceptible cultivar of sugar beet, canopy temperature correlated significantly with the density of the nematode parasite *H. schachtii* [80]. Kiwifruit trees infected by *P. syringae* pv. *actinidiae* were significantly warmer than the healthy ones, being localized in the outer canes of the canopy [48]. Temperature increases measured on *Dothistroma septosporum*-infected pines (causal agent of red band needle blight) and on *Zymoseptoria tritici*-infected winter wheat (causing septoria leaf blotch) could be positively correlated to the damages caused by these hemi-biotrophic fungi [40][81]. Necrotic spots on leaves from woody trees caused by three fungi (*Mycosphaerella cerasella*, *Elsinoe corni* and *Tubakia dryina*) could also be detected as dots with higher temperatures relative to healthy areas [82].

The fusion of thermography with other imaging techniques such as multi- or hyper-spectral reflectance or chlorophyll fluorescence imaging cameras is very often a more adequate approach for stress detection. The combination with chlorophyll fluorescence imaging allowed monitoring several constraints (fungal infection, galls and chewing damages caused by arthropods) in understory hardwood saplings. Thus, changes in transpiration could be related to photosynthesis impairment in affected trees [83]. However, the combination of TIR imaging with multi- or hyper-spectroscopy reflectance is more feasible since both techniques are based on passive measurements. Moreover, it is possible to obtain multiple reflectance VIs that could correlate with physiological parameters. Furthermore, VIs, together with temperature derived parameters, could be implemented on algorithms, to classify plants into categories of interest in

the earliest stages of infection. That was the case of important bacterial diseases causing high impact on both agriculture and environment, such as citrus greening or Huanglongbing in citrus trees [84], or *Xylella fastidiosa*, a quarantine pathogen, in olive orchards [85].

In the case of diseases caused by foliar fungal pathogens, the joint use of thermography and reflectance made possible to discriminate between severity levels. That was the case of red leaf blotch (caused by *Polystigma amygdalinum*) in almond trees [86] and also early and late leaf spot diseases (caused by *Passalora arachidicola* S. Hori and *Cercosporidium personatum*, respectively) on peanut trees [87].  $\Delta T$  correlated with disease severity caused by soil-borne fungal pathogens, such as *Verticillium dahliae* (causal agent of Verticillium wilt) in olive trees or *R. necatrix* in avocado trees. Moreover, classifying algorithms applied to the data obtained by spectral and TIR imaging could identify affected trees with high accuracy [72][88][89]. Minimum temperature corrected by air temperature could also be a good indicator of stress. This parameter showed a negative correlation with the normalized difference vegetation index (NDVI) in opium poppy orchards infected by the oomycete *Peronospora arborescens* (causal agent of downy mildew) [90]. Furthermore, a strong relationship between yield, several VIs and canopy temperature was observed in maize plants suffering tar spot complex (caused by *Phyllachora maydis* and *Monographella maydis*) [91], as well as in chickpea infected with *Ascochyta rabiei* (causing *Ascochyta* blight disease) [92].

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