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Full Length Article

Continuous Wavelet Analysis for Diagnosing Stress Characteristics of Leaf Powdery Mildew

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Abstract

Powdery mildew is a severe wheat disease that causes heavy yield loss all around the world. In order to identify and diagnose its early stress characteristics, the biochemical parameters and spectral data of wheat at the early infection stage were obtained, and then the chlorophyll-sensitive bands of the first, second and third leaf were selected using correlation coefficient method and continuous wavelet transform method. By comparing the determination coefficient and selected wavelength obtained from the two methods, we found that: 1) at the early infection stage, second leaf had highest reflectance value, followed by first leaf, and third leaf had lowest value; 2) when the original, first and second order data were processed by continuous wavelet analysis, the obtained chlorophyll-sensitive determination coefficients were 0.624, 0.685 and 0.704, respectively, which were 34.4, 8.4 and 9.1% higher than that obtained by correlation coefficient method, 0.280, 0.601 and 0.613, respectively. The selected wavelength, 885 and 1038 nm, was related with the biological characteristics of wheat leaf cell; 2188 nm was related with the moisture change within blade, which was reasonable. The results showed that continuous wavelet analysis is more promising than correlation coefficient analysis for the spectral diagnosis of powdery mildew.

Keywords: Powdery mildew; Spectral diagnosis; Correlation coefficient; Continuous wavelet; Chlorophyll

Introduction

Wheat powdery mildew is a major disease in the process of wheat growth in China. It can lead to the reduction of wheat spike and grain number, as well as blighted grain, resulting in a yield loss of 10−30% in general and over 50% in severe cases. During its prevention and treatment, excess spray of high-dose fungicide can pollute ecological environment. Thus, wheat powdery mildew has serious impacts on food security and farmland ecological environment in China (Qiao *et al.*, 2006; Zhang *et al.*, 2012).

Traditional diagnosis methods for wheat disease are time-consuming and laborious, with shorter time from early warning to prevention and treatment, which directly delayed the best disease prevention opportunity. To solve this problem, the remote sensing technology rapidly developed due to its advantages including secure, fast, real-time and large-area monitor. In recent years, scientists have used three scales of remote sensors, ground, low-altitude and aerospace, to perform related studies on warning and forecast of crop diseases (Malthus *et al.*, 1993; Cater *et al.*, 1994; Fletcher *et al.*, 2001; Apan *et al.*, 2004; Huang *et al.*, 2007). At present, the mechanism of spectral diagnostics for wheat crop powdery mildew has been preliminarily explored (Lorenzen and Jensen, 1989) and the related disease prediction model based on remote sensing has been established and applied and has achieved good economic and social effects (Qiao *et al.*, 2010). However, most current research uses correlation analysis to select sensitive bands and construct the remote sensing prediction model on this basis. The prediction accuracy of this method is higher in wheat peak incidence, but is lower at early infection stage (Qiao *et al.*, 2006). This is mainly related with the incidence characteristics of powdery mildew. That is, the disease first occurs at basal leaves of wheat; as the disease gradually progresses, it spreads upward; canopy spectral reflectance will have significant changes when the disease progresses to a certain extent. Although some methods (vegetation index methods, continuum removal methods and spectral characteristic parameters methods, etc.) have been applied in the spectrum diagnosis of pests and diseases, they all monitor the pathogenesis of disease stress and emphasize peak incidence (Malthus *et al.*, 1993; Adams *et al.*, 1999; Bravo & Moshou, 2003). Therefore, how to use remote sensing technology to improve the prediction at early infection stages and thus, to provide sufficient time for

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effective prevention and treatment of diseases is the difficult point and the purpose of our study.

Continuous wavelet analysis is a powerful tool for processing of spectral reflectance signal, and is commonly used in the extraction study for leaf biochemical parameter concentrations (Blackburn, 2007; Cheng *et al.*, 2010). It uses reflectance spectrum of leaf spectral to perform continuous wavelet transform, decomposes the spectral information into multiple scales of signal, then parses the characteristics of each scale signal, and finally indicates the differences of various biochemical components of leaves. Moreover, continuous wavelet analysis is used to study the chlorophyll and water content of leaves, indicating that this method has certain advantages over traditional methods and could get better results (Blackburn and Ferwerda, 2008; Cheng *et al.,* 2011).

In summary, this study tried to use correlation coefficient method and continuous wavelet transform method to select the sensitive bands from first, second and third leaves of wheat infected with powdery mildew. Comparison of determination coefficient and selected wavelength showed advantages of continuous wavelet analysis in disease diagnosis; in particularly, it provides a theoretical exploration for remote monitoring of wheat powdery mildew at early infection stages.

Materials and Methods

Experimental Design

The experiments were performed in February to April 2011 at the experimental farm of Beijing Academy of Agriculture and Forestry Sciences (39.93°N, 116.27°E). Experimental wheat was Jingdong 12, and managed with conventional fertilizer and water. Powdery mildew infection experiments were carried out by artificial inoculation. The inoculated bacteria were first cultured in pots in a greenhouse. When wheat seedlings got disease, they were inoculated to experimental area at the end of March.

Division of Stress Degree

This study focused on the impacts of powdery mildew on leaf spectrum and diagnosis method at early growth stage of wheat. Therefore, related studies were performed at the flagging stage of infection. At this point, the lower leaves of wheat showed obvious disease characteristics, and the upper leaves only had mild disease. Wheat disease level was divided referring to the plant pathology criteria (Agrios, 2004). At single leaf scale, it was divided into six levels according to the lesion percentage of total leaf area: level 0 (0%), level 1(<5%), level 2(<15%), level 3(<30%), level 4(<40%) and level 5(>=50%).Among them, level 1 and level 2 were referred to as mild disease, level 4 and level 5 were referred to as severe disease and level 3 was between mild and severe diseases. In order to provide theoretical support to the next layer canopy disease diagnosis, the first, second and third leaves were collected as experimental objects. Five samples were collected from each infection level, and totally 75 leaf samples were collected.

Data Collection and Processing

Leaf spectrum was determined by ASD spectroradiometer measurement (ASD FieldSpec® FR 2500, ASD Inc., Boulder, CO, USA). Five positions were measured on each leaf and five spectra were measured on each position. An average of 25 spectra was single leaf spectrum, and there were 75 leaf spectra in total. Reference whiteboard correction was performed before and after spectrometry, and leaf reflectance conversion referred to the methods described by Pu (2009). Furthermore, in order to eliminate the influence of other factors in experimental environment, we performed first and second order differential on the original reflectance spectra referring to the specific formula reported by Qiao *et al.* (2010).

The wavelength range of spectral data acquisition equipment, ASD spectroradiometer, was 350−2500 nm, the spectral resolution was 3 nm at 700 nm and 10 nm at 1500 and 2100 nm; the sampling interval was 1.4 nm at 350−1000 nm and 2 nm at 1000−2500 nm. To facilitate the analysis and processing, spectral intervals were all 1 nm, and the study scope was 400−2500 nm.

Biochemical Parameters Measurement

Chlorophyll content was measured using Dualex4 plant nitrogen balance index analysis instrument (Dualex®4, Force-A, French) as shown in Fig. 2. Three positions were measured and the average value represented the relative chlorophyll content for single leaf. This instrument can detect plant nitrogen deficit at early stage. Therefore, it has more advantages on probing plant stress compared to other instruments (Tremblay *et al.,* 2011).

Methods: The correlation analysis is used to study the potential dependencies among phenomena, and discuss the direction and relevance for specific phenomena with dependencies. It is a statistical method to study the correlation between random variables (Freedman, 2005).

Continuous wavelet transform (Eq. 1) is usually used to decompose a continuous function of time into wavelet. Compared to the Fourier transform, the difference of continuous wavelet transform is that it has a good time and frequency location when constructing time and frequency representation from processed signals (Cheng *et al*., 2010).

$$x(t)=\int_0^{\infty}\int_{-\infty}^{\infty}\frac{1}{a^2}X_{\omega}(a,b)\frac{1}{\sqrt{|(a)|}}\psi\left(\frac{t-b}{a}\right)dbda \quad (1)$$

Where, $\psi(t)$ is mother wavelet, a is an arbitrary positive real number, which represents the control of scale, and b is any real number, which represents the control of position.

Results

Different Levels of Leaf Spectral Characteristics under Disease Stress

When performing remote sensing monitoring on wheat powdery mildew canopy, the wind, light, background objects in external environment as well as the canopy structure of wheat itself could influence spectrum. In order to guarantee the accuracy of research, we chose single leaf, which had minimal impact factors, as experimental object. We first analyzed the spectral characteristics of leaves at different levels of disease, then used correlation coefficients and continuous wavelet analysis method to identify the chlorophyll-sensitive spectral band or internal and thus explore and resolve the construction of remote sensing model for characteristic leaves at different levels of disease. Fig. 3 was the original reflectance spectra of the infected leaves under different stress levels.

As shown in Fig. 3, the reflectance of infected leaves at various stress levels had significant difference in the 550−700 nm and 750−1250 nm spectral region. At 550−700 nm, the reflectance value of normal leaf (level 0) in Red Valley (670 nm) had minimal value, followed by mild leaves (level 1 and 2) and level 3 and severe leaves (level 4 and 5) had the highest value. They exhibited apparent three gradients, which were consistent with previous studies in other crop diseases. At 750−1250 nm, mild leaves had highest reflectance value, severe leaves (level 4 and 5) had lowest reflectance value, and normal and level 3 leaves had similar reflectance value. This indicates that when crops suffer from disease stress, the chlorophyll within leaves will change. This mainly occurs in visible and near infrared ranges. Especially near the Red Valley of visible light (670 nm), healthy leaves had lowest spectral reflectance value, and the reflectance value of disease leaves increased gradually as stress level enhanced. In the near infrared range, normal and severe leaves had great differences, while mild leaves had similar or higher than normal leaves. This is a self-regulation mechanism of crop to resist disease. That is, after infection, cell respiration enhances and chlorophyll activity increases, making the spectrum above or near normal.

On this basis, we performed comparative analysis on leaf spectral reflectance values for different leaf positions at leaves with same disease level and the results were shown in Fig. 4. The figure showed that the second leaf had highest reflectance value, followed by first leaf, and third leaf had lowest value. Comparative analysis revealed that the leaf reflectance values of different leaf positions at six levels all exhibited the trend as shown in Fig. 4. This is in line with the infection pattern at early growth stage of wheat growth (flagging stage). That is, incidence is from lower leaves, and third leaf had most severe disease; first leaf is new leaf with relatively weaker resistance, and it had mild disease; second leaf is fully developed with strong complete resistance, and

Fig. 1: Disease leaves of different levels

Fig. 2: Schematic diagram of Dualex4

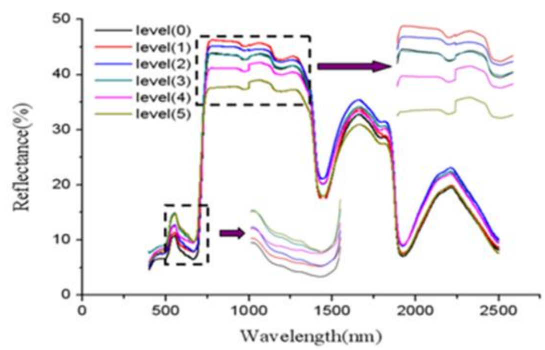


Fig. 3: Spectral characteristics under different stress level

it had mildest disease. This is also the mechanism of using spectrum to explore early disease prevention.

Chlorophyll-sensitive Band Selection using Correlation Analysis Method

When crops suffer from disease stress, accurate selection of characteristic sensitive bands of crop stress state is required to build disease prediction model. Based on the division of leaf position for disease leaves, the correlation analysis of leaf spectral reflectance and chlorophyll content of first, second and third leaves were shown in Fig. 5, which revealed the distribution of chlorophyll-sensitive bands. The chlorophyll-sensitive bands of first, second and third leaves were mainly in the range of 500−750 nm, displaying a highly significant negative correlation, with the absolute *r*

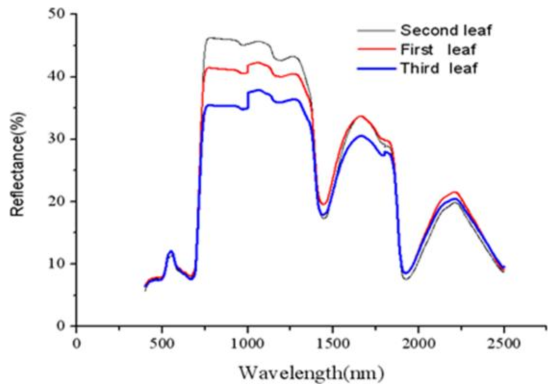


Fig. 4: Spectral reflectance of leaves from different leaf positions at leaves with same disease level

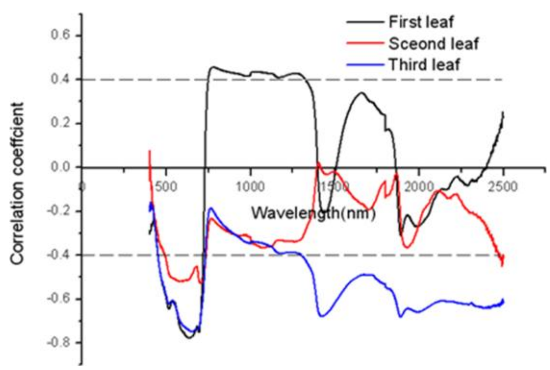


Fig. 5: Correlation analysis of leaf spectral reflectance and chlorophyll content of leaves from different leaf positions

value of higher than 0.4 (P>0.01); in addition, first leaf exhibited a significant correlation at 751−1300 nm, with the correlation coefficient *r* of near 0.4; third leaf showed a highly significant correlation at 1300−2500 nm, with the maximum of about 0.7; for second leaf, with the exception of 500−750 nm range, the leaf spectral reflectance and chlorophyll content had poor correlation in other ranges, suggesting the poor diagnosis outcome of correlation analysis on the chlorophyll of mildly infected leaves. For further analysis, we performed first and second order differential on original spectra, and used correlation analysis to identify sensitive range. As shown in Fig. 6, with the differential order increased, the noise of spectral signal was amplified. Here we only listed the correlation coefficient range with stable signal.

Fig. 6a showed that at different leaf positions, the leaf reflectance spectra and chlorophyll-sensitive bands were concentrated at 500−780 nm i.e., green, red and near infrared bands, which are the optimal bands for spectrum diagnosis of crop diseases. In addition, the correlation coefficient at near 550 nm reached maximum. The r value was around 0.8 for first and third leaves, and was only around 0.6 for second leave. In Fig. 6b, the red band was most sensitive to chlorophyll. The highest correlation coefficient of leaf spectrum and chlorophyll at different leaf

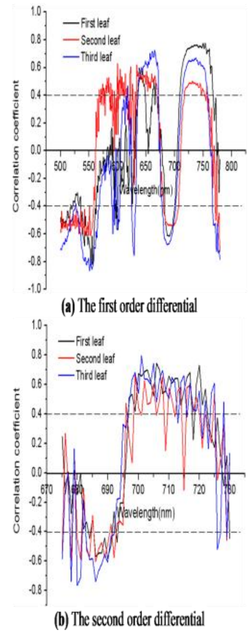


Fig. 6: Correlation coefficient of spectra and chlorophyll content of leaves from different leaf positions

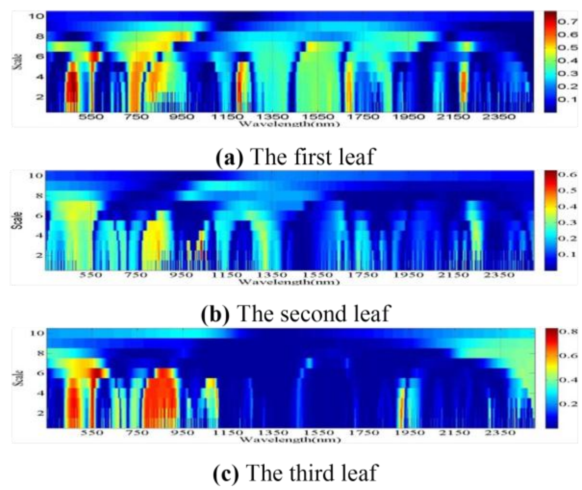


Fig. 7: Determination coefficients of spectra and chlorophyll content of leaves from different leaf positions using continuous wavelet analysis

positions was near 0.6, and the r value of second leaf was lower than first and third leaves. For above results, the first order differential was better than the second order differential.

Chlorophyll-sensitive Band Selection using Continuous Wavelet Analysis

Accurate selection of characteristic sensitive bands of crop stress state is required to build disease prediction model. Previous studies used characteristic parameters, vegetation index, continuum removal, normalization processing and

Table 1: Determination coefficient of chlorophyll content of leaves from different leaf positions and the selected wavelength (n=75)

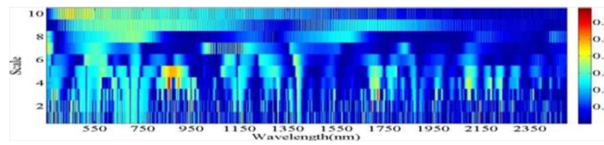
Spectra	Leaf position	Correlation coefficient		Wavelet analysis	
Original spectra	The first leaf	y=-611.1x+97.96	R^2=0.606 (636 nm)	y=13986x+47.78	R^2=0.781(475 nm)
	The second leaf	y=-73.96x+53.34	R^2=0.280 (705 nm)	y=7582x+62.97 1038	R^2=0.624(1038 nm)
	The third leaf	y=-239.0x+58.62	R^2=0.561 (652 nm)	y=19431x+41.78	R^2=0.828(484)
First order differential	The first leaf	y=-10993x+44.63	R^2=0.671 (553 nm)	y=63392x+12.59	R^2= 0.681(756 nm)
	The second leaf	y=-94839x+36.60	R^2=0.601 (883 nm)	y=-86100x +43.29	R^2=0.685(2188 nm)
	The third leaf	y=-11985x+32.88	R^2=0.755 (572 nm)	y = 69987x + 43.03	R^2=0.796(2317 nm)
Second order differential	The first leaf	y=-28117x+18.80	R^2=0.625 (750 nm)	y=7974.x+45.09	R^2=0.826(645 nm)
	The second leaf	y=51850x+31.73	R^2=0.613 (1444 nm)	y=9986x+17.02	R^2=0.704(885 nm)
	The third leaf	y=88450x+27.38	R^2=0.633 (701 nm)	y=10246.x+19.07	R^2=0.852(1615 nm)

other methods, but these methods are mostly used for the prediction of peak incidence. In order to identify methods to reflect the characteristics of disease crop at early onset, this study attempted to use the continuous wavelet transform method to decompose the spectrum into 10 scales of signal and to analyze the sensitivity of this method to disease stress.

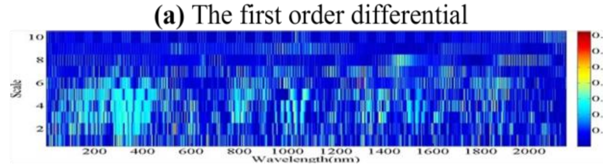
A histogram of original spectra of first, second and third leaves and chlorophyll-sensitive bands have been shown in Fig. 7. The figure showed that the chlorophyll-sensitive bands of first leaf were mainly distributed near 450 nm, 550 and 1100 nm, with the signals at 2-, 3- and 4-scale, and the determination coefficient of up to 0.78. The second leaf exhibited significant multiple correlation relationship near 1000 nm, with a determination coefficient of up to 0.624. The second leaf also exhibited a significant multiple correlation relationship, mainly concentrated at the 550 nm and 750−850 nm band ranges of 4-, 5- and 6-scale, with the highest determination coefficient of 0.83. The decomposition scale with high leaf spectral reflectance and chlorophyll content determination coefficient at different leaf positions was distributed at 2 to 6 scales, indicating that further decomposition of spectral signal can effectively improve the identification of chlorophyll-sensitive bands. In addition, compared to the traditional correlation coefficient method, the determination coefficient values at different leaf positions had improved significantly.

On the basis of the continuous wavelet analysis on original spectrum, we also carried out first and second order differential wavelet analysis. Since first and third leaves had better performance in the original spectrum analysis, they can be considered as obvious stage of disease stress and second leaf can be considered as early stage of infection. To illustrate the advantage of this method on the sensitive band selection at early stage, here only continuous wavelet transform figure of second leaf was listed.

The figure showed that with an increase in differential order, the spectral signal gradually weakened and the scope of chlorophyll-sensitive band gradually became narrow. At second order differential, only a single band left. This was consistent with the first and second order differential results at correlation coefficient analysis, i.e., most were noise signal and the effective signal range was getting smaller. However, the determination coefficient for second leaf was 0.624 originally, 0.685 at first order and 0.704 at second order; it effectively improved the chlorophyll-related determination coefficient. Selected wavelength included 1038 nm, 2188 nm and 885 nm, mainly distributed in the near infrared and mid-infrared ranges. Wavelets of 1038 and 885 nm were primarily related with the biological characteristics of the wheat; 2188 nm was primarily related with internal moisture change of blades. Therefore, there was a mechanism for selected wavelengths, and then the calculated determination coefficient was reliable.



(a) The first order differential



(b) The second order differential

Fig. 8: Coefficients of determination of spectra and chlorophyll content of second leaf using continuous wavelet analysis

Discussion

On the basis of disease data analysis using two methods, this study listed the determination coefficient and selected wavelength to further explain the advantages of continuous wavelet analysis. As shown in Table 1, for the original spectrum, continuous wavelet analysis was more effective than correlation coefficient analysis in improving the determination coefficient for chlorophyll prediction. Among them, first leaf improved 10%, and second and third leaves improved over 30%. Sensitive bands selected by continuous wavelet analysis were concentrated in the blue and near-infrared ranges, and the bands selected by correlation coefficient method were concentrated in red range. Although blue, green, red and near-infrared bands are all sensitive ranges to reflect the characteristics of chlorophyll stress, chlorophyll changes after disease stress did not rely solely on changes of a single band range, but rather on the results of synergistic changes of many intervals. Therefore, the multi-band can suggest that continuous wavelet analysis is more sensitive to chlorophyll concentration change under disease stress, as also proved by Zhang *et al.* (2012).

For the processing of first and second order differential, the determination coefficient obtained by continuous wavelet analysis were all higher than correlation coefficient, but it needs to be further proved whether this method can be used together with the first and second order differential. Because some selected wavelengths were at 1615 nm, 2188 nm and 2317 nm, which are far away from the traditional chlorophyll-sensitive range (Blackburn, 2007; Cheng *et al*., 2010). Related information indicated that these bands mainly reflect the plant water characteristics. When plants suffer from disease stress, besides chlorophyll, there will be significant changes in water (Huang *et al.*, 2004; Jiang *et al.*, 2007). Therefore, the wavelength ranges selected by continuous wavelet analysis are reasonable, but the stability requires in-depth studies in different diseases.

Wheat powdery mildew incidence is characterized by bottom-up, and it is difficult to diagnose at early onset using optical remote sensing (Lorenzen & Jensen, 1989; Qiao *et al.*, 2006). At present, researchers pay less attention to this issue (Qiao *et al.*, 2010). This study attempts to investigate the diagnosis mechanism at early disease stage in single leaf scale in order to provide theoretical support to the early remote sensing monitoring of powdery mildew at canopy level. According to the sample characteristics, third leaf had highest infection level, followed by first leaf, and second leaf had lowest level. The reasons include 1) the characteristics of incidence for bottom-up disease; 2) second leaf is functional leaf and is stronger than first and third leaves, resulting in the above sequence. Therefore, the second leaf can be considered as infected at lightest level. Correlation coefficient analysis revealed the existence of lowest determination coefficient, R^2 = 0.280 (705 nm), indicating that it was difficult to predict chlorophyll in second leaf. When using the continuous wavelet analysis, R^2 was 0.624 (1038 nm), and the determination coefficient increased by 34.4%. The processing results of first and second order differential both showed that continuous wavelet analysis effectively improved the determination coefficient (R^2 were 0.685 and 0.704). This suggested that continuous wavelet analysis had better results when dealing with leaves with insignificant disease characteristics.

In conclusion, after processing original, first order and second order data with continuous wavelet analysis, the derived determination coefficients for the chlorophyll-sensitive bands were 0.624, 0.685 and 0.704, respectively, which improved by 34.4%, 8.4% and 9.1% as compared to the values obtained by correlation coefficient, 0.280, 0.601 and 0.613. This proved that the method has a high application potential, especially for representing early disease characteristics. These results, however, included only 75 samples obtained from a single disease, whether some results were caused by noise and whether it can reasonably explain the disease changes need to be applied and proved using data from different diseases, crops and growth stages.

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