Pine wilt disease

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Pine wilt disease (PWD) is a major quarantine disease that causes the devastating death of Pinus species due to the pine wood nematode (PWN) Bursaphelenchus xylophilus (Steiner and Bührer) Nickle. Because of its rapid onset and spread in addition to the resulting high mortality, this disease is very difficult to control.

Keywords: pine wood nematode ; hyperspectral sensor ; drone ; forest monitoring ; pine wilt disease

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1. Introduction

Currently, the disease is widely distributed in eight countries, namely, in North America: the United States, Mexico, and Canada; in East Asia: China, Japan, South Korea, and North Korea; and in Europe: Portugal. However, globally, 52 countries have declared *B. xylophilus* as a quarantine pest and PWD as a quarantine disease [2,3,6,7,8,9,10,11,12]. Among the affected countries, China, Japan, and South Korea are the most severely impacted. In 1979, large numbers of pine trees died in Japan, with numbers of dead trees reaching as high as 2.43 million m^3 [13]. In South Korea, approximately 78.11 km^2 of pine forest was destroyed in 2005 [14]. Since PWD was first described in the Sun Zhongshan Mausoleum in Nanjing, China, in 1982, the disease has rapidly spread in the tropical and subtropical regions of China in only a few decades. In recent years, PWD has gradually invaded warm temperate zones and exceeded the 10 °C average annual temperature [15].

According to the Chinese Forestry Administration's No. 4 National Pine Wood Nematode Epidemic Area Announcement in 2019, PWD is widely distributed in 18 provinces [16]. Many trees have been destroyed, leading to large-scale deaths of pine trees, which has severely disrupted the ecological forestry environment and resulted in devastating losses. Because pine trees are environmentally friendly and valuable to the economy, the Chinese government, in addition to numerous others, attaches great importance to this resource, and have strengthened efforts toward the prevention and control of PWD, especially in recent years. Our group has done a lot of work in this field. A wood sampling method was reported in 2010 [17]. It can estimate nematode numbers rapidly in the wood of *Pinus massonina* with only 10 min. In 2011, our group developed a rapid detection of *B. xylophilus* in stored *Monochamus alternatus.* This method can detect nematodes by PCR amplification [18]. One of the key strategies in combatting PWD is the early identification of epidemic areas via large-scale forest monitoring. PWD symptoms can be leveraged for the intelligent monitoring of forest pines [4]. Large-scale forest monitoring of pine wilt disease can be achieved by hyperspectral techniques.

Hyperspectral techniques can be used for object identification and object character analysis. It is widely used in agriculture, oceanography, geology, atmosphere, and environmental sensing [19]. In agriculture, hyperspectral techniques can be used for pest and disease monitoring [20,21,22,23,24,25], biomass estimation [26], and biological stress monitoring [27]. In the field of pest and disease monitoring, it has been applied to many plants, including tomatoes, grapes, and wheat [20,21,22]. Similarly, it can also be applied to the early monitoring of pine wilt disease. However, because hyperspectral techniques are not mature, there are a lot of problems and difficulties that limit its practical applications.

2. PWD Symptoms

PWD is generally transmitted by insect vectors such as *M. alternatus*. When insect vectors feed on pine trees, the pine wood nematode parasite is transferred to pine trees, and PWD caused by the nematode is generally reflected in the canopy of the host plant. PWD is generally divided into three stages on the basis of its symptoms [28]:

(1) The difference between a healthy plant and plant during an early disease stage cannot be observed with the naked eye. The duration of this early stage is mainly affected by pine age and species, environmental temperature, and pine wood nematode virulence. Pine wood nematodes begin to multiply in the xylem of pine trees, blocking the internal ducts of pine trees. Pine trees cannot perform normal water metabolism, and pine resin secretion is reduced along with slight

changes in chlorophyll and carotene contents. Clevers' research showed that the red-edge chlorophyll index is linearly correlated with the canopy chlorophyll content [29]. In addition, Köksal found that the normalized difference vegetation index is strongly associated with the carotene content of leaves [30]. Therefore, the change in chlorophyll and carotene content can be detected by hyperspectral remote sensing.

(2) In the middle stage of the disease, pine tree resin secretion completely stops, and the transpiration effect is weakened. Needles in the crown of the tree gradually become yellowish. Visual differences between healthy and infected pines are evident at this stage. The wilting appearance of the pine during this period differs from that of the healthy pine. PWD causes pine needles to wilt from the inside to the outside.

(3) At the end stage of the disease, needles on the crown of the tree turn brown or reddish brown. The whole plant dies, and the needles do not fall that year.

The spectral characteristics of pines vary according to the type of stress. Different spectral indices can be used to build models to identify PWD and differentiate it from other decline agents, such as bark beetles, defoliating fungi, or even severe droughts [31].

3. Traditional PWD Monitoring Technology

3.1. PWD Pine Tree Monitoring Methods

PWD diagnosis depends on human reconnaissance and visual rating (Figure 1), followed by the isolation of nematodes found in diseased wood. Microscopy is used to observe the morphological characteristics of the nematodes and determine whether PWNs are present in the pine tree. Traditional methods of morphological identification require training for recognizing nematodes. Molecular identification is also available but is expensive for large-scale monitoring [32].

Figure 1. Pine seedlings: healthy plus five different infection levels.

In certain harsh conditions that make it difficult to enter a forest, the transmission source cannot be treated in time after pine trees are infected with nematodes, resulting in large-scale damage to the pine forest. In addition, the field diagnosis of PWD based on the analysis of visual symptom is subject to variation due to the different evaluators, and it is easy to draw incorrect conclusions [33,34,35,36]. Many researchers have developed DNA-based methods to rapidly identify PWD using molecular biology methods [37,38,39]. For example, a sampling-staining-PCR detection workflow system was established for the first time by Wang and colleagues [40]. This system can detect pine wood nematodes directly from infected *P. massoniana* within 5 h without requiring nematodes to be isolated from samples. However, this method does not address the problem of allowing pine wood nematode diagnosis before disease symptoms appear (Figure 1).

3.2. Pine Wood PWD Monitoring Technology

PWD can be monitored with the “punching and flowing resin” method since this disease leads to a reduction in resin secretion from pine trees. Because this method is not specific to PWD, it was gradually eliminated [41]. On the other hand, PWD can also be diagnosed by monitoring the density of *M. alternatus*. *M. alternatus* is abundant in pine forests, but this insect does not always transmit the PWN responsible for PWD. In fact, its odds of carrying PWN range from 0 to 60%, and few of these PWNs successfully invade tissue. Therefore, monitoring PWD on the basis of *M. alternatus* is ineffective [42].

Traditional methods for monitoring PWD are time-consuming and labour-intensive. This disease can be diagnosed with certainty by nematode separation and morphology identification. Therefore, an efficient method is urgently needed for the intelligent monitoring of PWD.

4. Principles of Monitoring PWD with Hyperspectral Remote Sensing Technology

The advantage of using hyperspectral remote sensing technology in disease classification is that it can combine image information and spectral information. Image information can be used to analyse surface quality features such as sample size, profiles, and defects. Because spectral absorption varies from one component to another, an image reflects a certain feature at characterised bands, and spectral information can fully reflect a discrepancy in the physical structure and chemical composition of a sample [43]. These characteristics determine the unique advantages of spectral imaging technology in testing the internal and external quality of farm products. Spectral imaging technology makes full use of the absorption or radiation characteristics of a substance in different electromagnetic spectra. One-dimensional spectral information is added on the basis of ordinary two-dimensional space imaging. Due to differences in the composition of various substances, differences also exist between their corresponding spectra such that the spectrum of the target can be used in identification and classification. To achieve monitoring, plant diseases are identified through various parameters, such as morphological changes, temperature changes, transpiration rates, and volatile organic compound release by pathogen-infected plants [44]. The delicate process of host–pathogen interactions also affects the optical characteristics of plants to a certain extent [45]. Data about these changes can be obtained by sensors. Hyperspectral sensors offer the most abundant data information. This technology can supply sufficient evidence, demonstrating its feasibility for the early detection of plant diseases. With the development of hyperspectral technology in recent years, increasing numbers of researchers have applied it to plants, including for plant-nutrition monitoring [46], the automatic classification and monitoring of plant diseases [47,48], and their early detection [45], as well as to obtain digital phenotypes for disease-resistant plant breeding [49,50]. For example, pine wilt disease was diagnosed using hyperspectral methods by Kim's group. In their research, significant differences were observed in vegetation indices such as the normalized difference vegetation Index (NDVI), the green normalized difference vegetation index (GNDVI), the plant senescence reflectance index (PSRI), and the pigment specific normalized difference (PSND), beginning on 20 August 2012 [51]. Our group study the hyperspectral characteristic of healthy and withered masson pine, and Figure 2 shows the research results. If a plant is infected with PWD, the green needles of the pine trees begin to change colour. The spectral reflectance of pines varies over the different degrees of infection. Therefore, disease level can be evaluated on the basis of spectral reflectance.

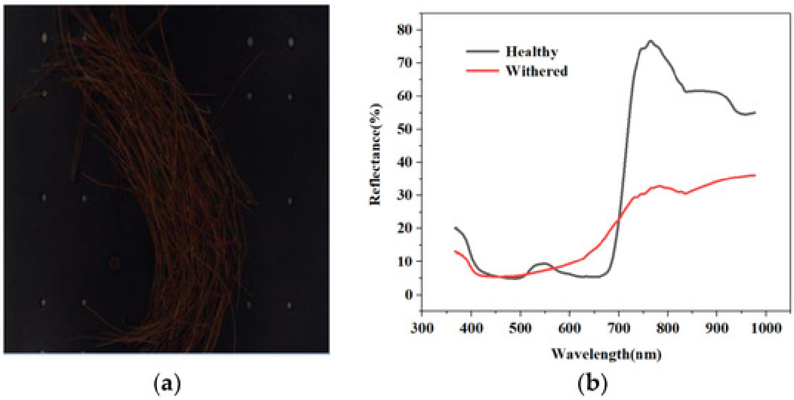

Figure 2. Detection of pine wilt disease using hyperspectral techniques (**a**) Hyperspectral images of diseased pine needles (**b**) Hyperspectral reflectance of healthy and withered masson pine.

Spectral imaging technology can collect many narrow and continuous hyperspectral reflectance datasets in the ultraviolet, visible, near-infrared, and mid-infrared regions of the electromagnetic band, providing complete and continuous spectral curves for each pixel. Imaging spectrometers can be divided according to their resolution, as follows [52].

Multispectral imagers (MSIs): the obtained target has a wavelength band between 320 and 1300 nm, and the spectral resolution is generally close to 100 nm; this technology is mainly used in zone classification [53].

Hyperspectral imagers (HSIs): the obtained target band is between 350 and 2500 nm, and the spectral resolution is approximately 10 nm; this technology is widely used in various fields, such as food safety, quality, and biomedical agricultural product testing [54].

Ultrahyperspectral imagers (USIs): the obtained target band is between 1000 and 10,000 nm, and the spectral resolution is below 1 nm; this technology is commonly used when fine detection is required, such as in the case of atmospheric particle detection [55].