Research Article
Infection Trace of Pine Wilt Disease Based on Terahertz Spectroscopic Technology

[1]Ling Jiang, [1]Chun Li, [2]Lin Huang, [3]Zhenwei Zhang, [3]Cunlin Zhang and [1]Yunfei Liu
[1]College of Information Science and Technology,
[2]Forest Resources and Environment Institute, Nanjing Forestry University,
159 Longpan Rd., Nanjing 210037, Jiangsu, China
[3]Terahertz Laboratory, Department of Physics, Capital Normal University,
The West Third Ring Road, Huayuan Bridge, Beijing, China

Abstract: In this study, we present the terahertz spectroscopic features of pine needle infected by pine wilt disease during different infection stages by applying terahertz spectroscopic techniques. We measure the absorption variation of the pine needle samples with infection time by Terahertz Time Domain Spectroscopy (THz-TDS). The absorption intensity in the frequency region of 0-2.5 THz increases gradually with infection time. While the pine wood is infected above four months, the absorption intensity reaches to maximum. However, the calculated relative refractive index compared with the reference signal of PET powder reduces with infection time. The higher frequency measurement by Fourier Transform Infrared spectroscopy (FTIR) doesn't show the absorption variation of the samples due to huge signal attenuation in the transmission path of the samples. This study demonstrates that the relation of absorption intensity and refractive index of the pine needle sample with the infection time. We can trace the infection extent of the pine wood by measuring its terahertz spectroscopic property.

Keywords: Absorption, infection time, pine wilt disease, refractive index, terahertz spectroscopy

INTRODUCTION

In forest ecosystems the Pinewood Nematode (PWN), *Bursaphelenchus xylophilus* is considered one of the most important pests and pathogens in the world. The general fear of establishment of the PWN, the causal agent of the Pine Wilt Disease (PWD), into countries where conifer forests assume great importance, stems from the devastating damage caused by this nematode to pine forests. The economic importance posed by the PWN clearly reinforced the need for an accurate diagnosis of the species, where morphological studies remain the standard method for routine identification (Ryss *et al*., 2005). Yet, an integrated morphological identification system to all the species of the genus has been lacking. Furthermore, the fact that more than 70% of these species occur in pine trees makes the identification even more uncertain. Due to the difficult identification and constrains of morphological observations between *Bursaphelenchus* speci*es*, alternative molecular tools have become a valuable instrument for species and sub-specific separation (Hotchkin and Giblin, 1984). Currently the conventional molecular method is real-time

fluorescence Polymerase Chain Reaction (PCR) technique, which focus on the investigation of the PCR segment of Deoxyribose Nucleic Acid (DNA) molecule of the *Bx* and the *Bm* samples by introducing the fluorescence marker (Cao *et al*., 2005; Wang *et al*., 2007). This method has several defects, for instance, time-consuming, high cost, relative high error rate due to the fluorescence marker. It's mainly employed for quarantine purposes. To early diagnose the pine wilt disease, it's necessary to find a fast and exact method. Terahertz spectroscopic technology has been successfully used to biology and medicine to study the animal and plant protein, nucleic acid and amino acid and so on (Fischer *et al*., 2002; Siegel, 2004; Kutteruf *et al*., 2003). However, the spectral property of DNA has not been investigated thoroughly due to its huge mass (Markelz *et al*., 2000; Liu *et al*., 2009a, b, 2010). In this study, we study the absorption characteristics of the pine needle infected by the pine wilt disease during different infection stages by using Terahertz Time Domain Spectroscopy (THz-TDS) and Fourier Transform Infrared spectroscopy (FTIR). The pine needle samples are more easily obtained in contrast to the preparation of DNA of pine wood nematode, which

increases largely the detection efficiency. In addition, the ingredients of pine needle are easily analyzed by quantum chemical method (i.e., Gaussian 09 software). We obtain the different absorption characteristic of the pine needle samples. The experimental results will be presented in this study.

MEASUREMENT SETUP AND METHODS

The THz spectroscopic characterization of the pine needle samples with different infection time are performed by the THz-TDS and the FTIR spectroscopy, respectively. By measuring the change of the intensity of probe beam, we can obtain the THz wave signal which carries the time domain and frequency domain information of the sample in the frequency range of 0-2.5 THz.

Figure 1 shows the schematic of THz-TDS measurement system. The THz time domain spectrometer employs a Spectra-Physics tunable sapphire laser (MaiTai laser). These pulses are centered at 810 nm with approximately 980 mW of average output power. Its output beam is then fed into the sapphire laser system to generate 100 fs pulses at an 82 MHz repetition rate. Once the 810 nm pulsed beam has left the laser, it is split by a 90/10 beam splitter to create two beams: pump and probe. Receiving most of the laser power, the pump beam then goes to a delay stage. The beam is passed twice over the stage to allow for a maximum time delay scan. After passing through the stage, the pump beam is then incident on the THz InAs emitter.

When the pulsed pump beam is incident on the THz InAs emitter, a coherent THz pulsed beam is generated. The THz radiation is collected by two effective focal length Off-Axis Parabolic (OAP) mirrors. This collimates the THz beam and directs it to the sample. The transmitted radiation is then collected by another two OAP mirrors that refocuses the beam back to the same size on the ZnTe detector crystal. The

sample is mounted on an actuator system to allow it to slide in and out of the THz beam path. The probe beam passes through several mirrors and a Glan-Laser polarizer P, then is reflected off a pellicle membrane and focused on the ZnTe crystal. The pellicle is transparent to the THz pump beam, but reflective for the 810 nm probe. The probe beam is then circulary polarized by a quarter-wave plate and is split by an analyzer PBS, finally detected by a pair of balanced photodiodes. The signal is recovered by a digital lock-in amplifier and recorded on the computer. The THz system is built inside a temperature controlled and nitrogen purged box.

In order to understand the spectral property of samples at higher frequencies above 2.5 THz, we measure its absorbance by using Fourier Transform Infrared spectroscopy (FTIR), which is a technique used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. The conventional FTIR system consists of optical source, Michelson Interferometer and detector (Carl, 1997; Mankova *et al.*, 2012). In our measurement, we employ the FTIR spectrometer of Vertex 80 made by Bruker Company to measure the transmission spectral performance of the pine needle samples.

The five pine needle samples with healthy, infected with 20 days, 2 months, 4 months and dead naturally were measured by the THz-TDS and the FTIR spectroscopy respectively. The pine needle samples are dried by vacuum drying oven until the water content is below 5% and crushed into powder. It is mixed with Polyethylene (PET) powder and compressed into a thin pill under high pressure. The final pill has a 1-mm thickness, 13-mm diameter and 100-mg weight. The pure PET powder is also made into a pill with same thickness and weight as the reference signal, which is transparent for THz signal.

In the THz-TDS measurement, The refractive index $n_s(\omega)$, absorption coefficient $\alpha_s(\omega)$ and
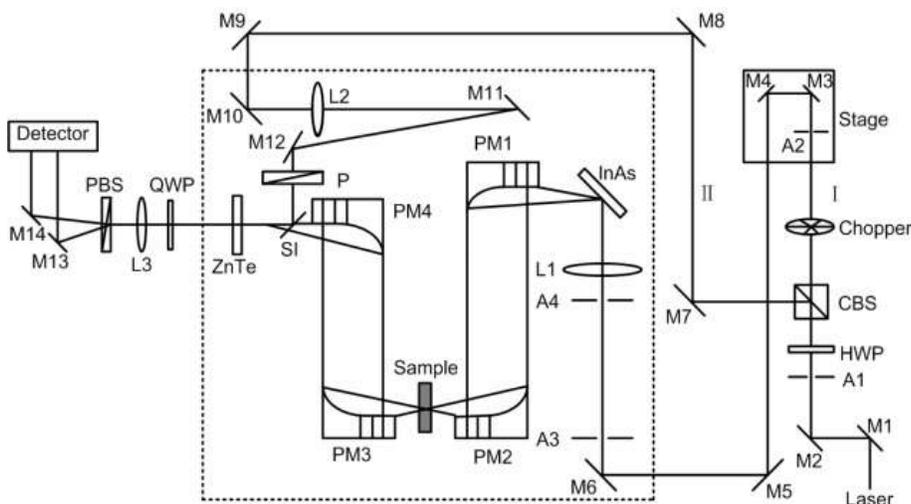

Fig. 1: Schematic of THz-TDS measurement system

extinction coefficient $k_s(\omega)$ of the pine needle samples are obtained by means of the physical model developed by the research groups (Timothy *et al*., 2001; Duvillaret *et al*., 1999). Assuming that $E_{THz}(\omega)$ is the incident field, $E_{ref}(\omega)$ the reference field and $E_{sam}(\omega)$ the field where the signal passes through the sample. The reference field can be given by:

$$E_{ref}(\omega) = E_{THz}(\omega)e^{-j\tilde{n}(\omega)\omega L/c} \tag{1}$$

where,

L = The distance where the reference wave propagates in linear (uniform) medium

c = The velocity of light

When the THz wave incidence on the sample vertically, based on the Fresnel's equations the functions of transmission coefficient $t_{os}(\omega)$ can be written as:

$$t_{os}(\omega) = 2\tilde{n}_s/(\tilde{n}_0 + \tilde{n}_s) \tag{2}$$

where, $\tilde{n}_s$ and $\tilde{n}_0$ is the complex refractive indices of the sample and the medium around the sample respectively, with the expression of $\tilde{n} = n(\omega) - j\kappa(\omega)$.

For thick samples with the thickness of the order of millimeter, the Fabry-Perot effect can be ignored. Therefore, the field $E_{sam}(\omega)$ passing through the sample is:

$$E_{sam}(\omega) = E_{THz}(\omega)t_{os}e^{-j\tilde{n}_s(\omega)\omega d/c}t_{so} \tag{3}$$

where, d is the thickness of the sample.

In our experiment, the refractive index $\tilde{n}_0$ is approximately 1. Substituting Eq. (2) into (3), the ratio of $E_{ref}(\omega)$ to $E_{sam}(\omega)$ is given by:

$$\begin{aligned} H(\omega) &= E_{sam}(\omega) / E_{ref}(\omega) \\ &= 4\tilde{n}_s(\omega)e^{-j\omega d(\tilde{n}_s-1)/c} / (1+\tilde{n}_s)^2 \\ &= \rho(\omega)e^{-j\varphi(\omega)} \end{aligned} \tag{4}$$

where, $\rho(\omega)$ and $\phi(\omega)$ are the ratio of the amplitude and the phase difference between the sample and the reference which are obtained from the THz-TDS experiment. Considering $\kappa_b \ll n_b$, we obtain:

$$\rho(\omega) = 4n_s(\omega)e^{-\kappa_s(\omega)d\omega/c} / (n_s(\omega)+1)^2 \tag{5}$$

$$\varphi(\omega) = (n_s(\omega)-1)\omega d / c \tag{6}$$

Finally, the expressions of the refractive index $n_s(\omega)$, the absorption coefficient $\alpha_s(\omega)$ and the extinction coefficient $k_s(\omega)$ can be achieved as follows:

$$n_s(\omega) = \varphi(\omega) \cdot \frac{c}{\omega d} + 1 \tag{7}$$

$$\alpha_s(\omega) = \frac{2}{d} \cdot \ln(\frac{4n_s(\omega)}{\rho(\omega) \cdot (n_s(\omega)+1)^2}) \tag{8}$$

$$k_s(\omega) = \frac{\alpha_s \cdot c}{2\omega} = \frac{c}{\omega d} \cdot \ln(\frac{4n_s(\omega)}{\rho(\omega) \cdot (n_s(\omega)+1)^2}) \tag{9}$$

It should be noted that the real refractive index and absorption coefficient are relative values after correcting the absorption impact of the reference samples of the PET powder.

RESULTS AND DISCUSSION

We measure the transmission characteristics of the pine needle samples with different infection time by using THz-TDS spectrometer. The measurement results in time domain show different time delay for the five samples, as shown in Fig. 2. We calculate the absorption coefficient and refractive index of the pine needle samples after correcting the affect of the PET powder as the reference signal according to Eq. (7) and (8), as shown in Fig. 3 and 4. The transmission loss of the THz signal through the pine needle samples increases with the infection time. While the pine tree is infected by over 4 months, the absorption characteristic is similar to that of the naturally dead sample, which approaches the largest value. It means the pine tree is dehydrated and the transpiration decreases drastically resin secretion and stop in the dead stage. The calculated relative refractive index compared with that of PET powder reduces gradually when the pine tree is infected further with time. The relative refractive index is close to 1.0 when the pine tree dies completely.

The measurement by THz-TDS indicates the pine needle samples have different absorption features with time in the frequency range of 0-2.5 THz. Since the absorption coefficient and the refractive index have disciplinarian variation with infection time, we can trace the infection time of the pine tree by observing the increase of the absorption or the reduction of the relative refractive index. The absorption in Fig. 4 show some ripples over the entire frequency range, but obvious specific frequency peak doesn't appear. It is probably because the pine needle sample is the combination of various ingredient, such as coloring matter, cuticle, cellulose, protein and so on. The THz resonance frequency peaks are hided by the complicated ingredient. In order to find the specific frequency peak, it is necessary to distill the single component, for instance chlorophyll a and b. This study will be done in the future experiment.
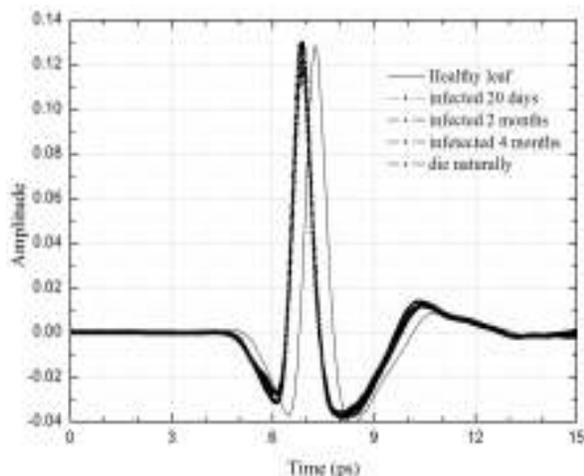
Fig. 2: Amplitude as a function of time for the pine needle samples with healthy, infected by 20 days, 2 months, 4 months and dead naturally, measured by THz-TDS
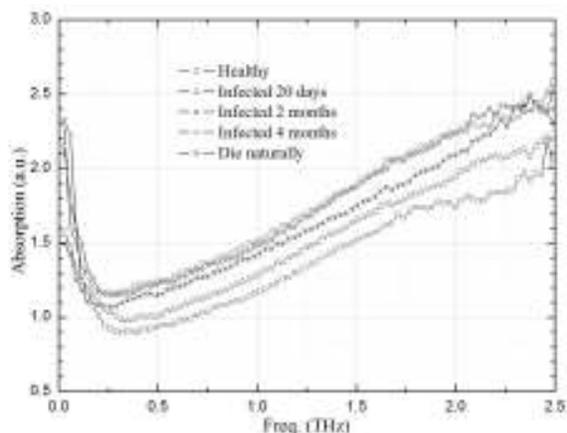

Fig. 3: Absorption coefficient as a function of frequency for the pine needle samples with healthy, infected by 20 days, 2 months, 4 months and dead naturally
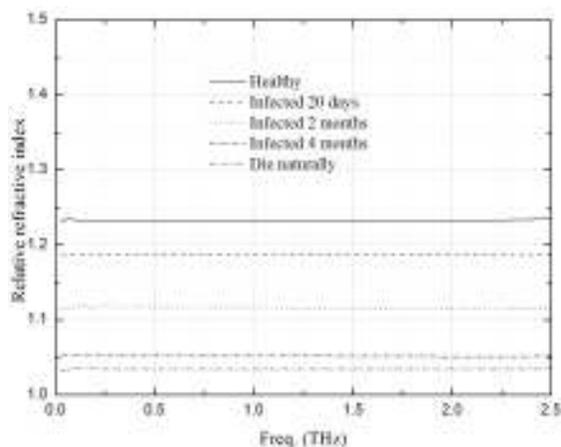

Fig. 4: Relative refractive index as a function of frequency for the pine needle samples with healthy, infected by 20 days, 2 months, 4 months and dead naturally

We measure the transmission response above 1 THz by using broadband FTIR spectroscopy. In the frequency range of 1-10 THz, the PET powder sample has a sharp absorption peak around 3.4 THz. The measured FTS (Fourier Transform Spectroscopy) responses exhibit random fluctuations and very low transmission for the five pine needle samples. It means most THz signal is absorbed by the samples due to complicated components.

CONCLUSION

We have measured the time domain and frequency domain responses of the five pine needle samples by using the Terahertz Time Domain Spectroscopy (THz-TDS) and the Fourier Transform Infrared spectroscopy (FTIR). The absorption coefficient and the refractive index are obtained by TDS measurement results, which exhibit disciplinarian variation with the increase of infection time. The infection time of the pine tree can be detected by observing the variation trend of the absorption coefficient and the relative refractive index. Further measurement of the absorption characteristics at higher terahertz frequencies above 1 THz is made by the FTIR, unfortunately the specific frequency peak doesn't appear due to the huge absorption of the samples. In summary, we can trace the infection time of the pine needle samples by observing the absorption intensity and the refractive index of the samples at 0-2.5 THz. This investigation will provide a new method based on terahertz spectroscopy technology to diagnose the pine wilt disease and trace the infection time.

ACKNOWLEDGMENT

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