

DETECTION OF TREE FREEZE–THAW STATUS BASED ON THE INTEGRATION OF STEM WATER CONTENT AND STRATIFIED TEMPERATURE

结合茎干水分和分层温度的林木冻融状况检测研究

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ABSTRACT

In most mid-to high-latitude regions, trees are frequently subjected to severe freeze-thaw stress during the overwintering period, yet accurately detecting their freeze-thaw status remains challenging. In this study, *Malus spectabilis*, a common ornamental tree species in northern China, was selected as the research subject. A self-developed stem water content sensor based on the standing wave ratio (SWR) principle, in combination with a miniature thermocouple array, was employed to achieve in situ and non-destructive monitoring of internal stem water dynamics and radially stratified stem temperature. Furthermore, an Internet of Things (IoT)-based plant freeze-thaw monitoring system was established. The temporal variation characteristics of these parameters during overwintering were analyzed, on the basis of which a computational model of the freeze-thaw process was developed, and a novel method for detecting stem freeze-thaw dynamics was proposed. The results revealed that alternating freeze-thaw cycles in stems are accompanied by distinct endothermic and exothermic phenomena, with the freezing and thawing processes progressing radially from the outer to the inner stem layers—allowing the migration trajectory of freeze-thaw peaks to be tracked. In addition, different types of plant fiber materials were applied to stem tissues to verify the effectiveness of cold-resistance measures. This study provides new insights into the mechanisms regulating tree cold hardiness during overwintering and offers practical references for the scientific management of trees in cold regions.

摘要

在中高纬度地区，树木在越冬期间常受到严重的冻融胁迫，但其冻融状态的准确检测仍具有较大挑战性。本研究以北方常见的观赏树种海棠（*Malus spectabilis*）为研究对象，采用自主研发的基于驻波比（SWR）原理的茎干水分传感器，结合微型热电偶阵列，实现了茎干内部水分动态及径向分层温度的原位、无损监测。同时，构建了基于物联网（IoT）的植物冻融监测系统。通过对越冬期间上述参数的时序变化特征进行分析，建立了茎干冻融计算模型，并提出了一种新的茎干冻融动态检测方法。结果表明，茎干内交替的冻融循环伴随着明显的吸热与放热现象，冻融过程沿径向由外向内推进，可实现冻融峰迁移轨迹的动态跟踪。此外，本研究还采用不同类型的植物纤维材料包裹茎干组织，以验证防寒措施的有效性。本研究为阐明树木越冬期的抗寒调控机制提供了新的认识，也为寒冷地区树木的科学管理提供了实践参考。

INTRODUCTION

In northern China and other regions at similar or higher latitudes, trees experience their greatest physiological stress during the non-growing season as a result of low-temperature conditions (Larran et al., 2023). During the overwintering period in particular, ambient temperatures frequently decline to their annual minimum, giving rise to freeze-thaw phenomena within stem tissues that play a crucial role in plant water transport and physiological regulation (Cheng et al., 2021; Zhao et al., 2021). These alternating freezing and thawing processes may cause xylem conduit rupture due to the volumetric expansion of water upon freezing or lead to embolism formation that diminishes hydraulic conductivity (Maruta et al., 2022; Li et al., 2023).

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At the microscopic scale, intracellular ice crystallization can induce severe structural damage to plant tissues and organs (Bozonnet *et al.*, 2024). Such injuries directly influence whether the water transport capacity of the stem can recover in the subsequent growing season and, consequently, whether normal tree growth can resume (Lintunen *et al.*, 2022; Dai *et al.*, 2023). Therefore, implementing effective cold-resistance strategies during overwintering and accurately assessing the freeze-thaw status of stems are of considerable practical and ecological importance.

Early studies on freeze-thaw phenomena in trees primarily employed histological sectioning to determine whether water in detached leaves, stems, or branches had frozen. However, this approach inevitably caused damage to the trees themselves (Charra-Vaskou *et al.*, 2023; Mucchiani *et al.*, 2024). Subsequently, researchers introduced more advanced techniques and precise instruments, such as low-temperature scanning electron microscopy, thermal infrared imaging, and ultrasound, which allowed for non-destructive and accurate analysis of freezing and thawing in stem tissues (Ameglio *et al.*, 2001; Stegner *et al.*, 2020). Nevertheless, these methods are expensive and have limited applicability, making them unsuitable for long-term monitoring in harsh field environments (Xu *et al.*, 2024). Recently, some researchers have begun to use water content sensors based on principles such as time domain reflectometry (TDR), frequency domain (FD), and standing wave ratio (SWR) to monitor freeze-thaw dynamics of stem tissues under complex field conditions (Wang *et al.*, 2017; Sun *et al.*, 2019; Tian *et al.*, 2023). However, TDR sensors require probes to be inserted into stem tissues, which may shift during freezing and thawing, thereby affecting measurement accuracy (Nadler *et al.*, 2003; Wang *et al.*, 2017). In contrast, water sensors based on FD and SWR principles enable non-destructive and in situ monitoring of freeze-thaw processes in stem water content during overwintering (Sun *et al.*, 2019; Gao *et al.*, 2021). For instance, Xu *et al.* (2024) applied an SWR-based stem water content sensor to characterize seasonal water dynamics and influencing factors during overwintering. Similarly, Zhao *et al.* (2021) employed an LWFTD sensor to successfully capture dynamic changes in freeze-thaw status and ice content in poplar stems.

Although previous studies have provided valuable insights into the alternation of freezing and thawing in stem tissues during overwintering, few have incorporated another crucial determinant—stem temperature—into freeze-thaw monitoring. How does solid ice propagate within stem tissues during freezing and thawing? Are the cold-resistance measures commonly applied to tree stems during overwintering truly effective? To address these questions, *Malus spectabilis*, a widely cultivated ornamental tree species in northern China, was selected as the research subject. A self-developed in situ stem water-content sensor based on the standing-wave-ratio principle, together with a miniature thermocouple array, was employed to obtain and analyze the variation characteristics of stem water content and stratified stem temperature during the overwintering period. In combination with Internet of Things (IoT) technology, a real-time monitoring system for plant freeze-thaw conditions was also established. Furthermore, different types of cold-resistance materials were applied to the stem tissues to assess their protective effectiveness. On this basis, a computational model of the freeze-thaw process was developed, and a detection method for stem freeze-thaw dynamics was proposed, aiming to comprehensively elucidate the mechanisms of freeze-thaw behavior in stem tissues, track changes in ice content and its migration trajectory, and provide new insights into water transport and freeze-thaw regulation in trees during overwintering.

MATERIALS AND METHODS

Study site and materials

The study was conducted at the Sanqingyuan Nursery of Beijing Forestry University, located in Haidian District, Beijing, China (116°21'14'E, 40°0'54'N; approximately 50 m above sea level). The soil at the site is classified as clay loam with a pH ranging from 7.0 to 8.0. The regional climate is characterized as a warm temperate, semi-humid, and semi-arid continental monsoon type (Xu *et al.*, 2024). The area receives approximately 2,560 hours of sunshine annually, with an average annual evaporation of about 1,800 mm.

Four *Malus spectabilis* trees - a common broad-leaved species widely used in urban landscaping in northern China - were selected as experimental subjects. The selected trees exhibited straight stems, vigorous growth, and no signs of disease or pest damage, with an average height of 3.0 m and an average diameter at breast height (DBH) of 6 cm. During the non-growing season, the stems of these trees were wrapped with plant fiber materials of varying thicknesses to evaluate the effectiveness of stem wrapping as a cold-resistance measure (Figure 1).



Fig. 1 - Malus samples with different types of frost protection treatments

Monitoring of plant freeze-thaw status

Stem temperature is indirectly related to the physiological status of woody plants, and using temperature alone to assess freeze-thaw conditions may introduce inaccuracies. Moreover, previous studies have demonstrated that freeze-thaw dynamics are closely associated with variations in internal stem water status (Li *et al.*, 2024; Xu *et al.*, 2024). Therefore, in this study, stem temperature and internal stem water dynamics were monitored concurrently, and a real-time monitoring system for plant freeze-thaw conditions was established. This integrated approach provides a robust data foundation for a more comprehensive analysis of plant freeze-thaw status and for subsequent estimation of stem ice content. The following section describes in detail the methods used to measure stem water content and temperature, as well as the architecture and deployment of the monitoring system.

Measurement of stem water content

In this study, a self-designed stem water content sensor based on the standing wave ratio (SWR) principle was employed to achieve real-time and non-destructive monitoring of internal stem water dynamics (volumetric water content) during the experiment. The sensor consists of a 100 MHz high-frequency active crystal oscillator, a 50Ω coaxial transmission line, a wave detection circuit, an amplification circuit, dual 304 stainless-steel detection rings, and a protective casing. The schematic diagram of the sensor is shown in Figure 2.

The 100 MHz signal source generates a high-frequency electromagnetic wave that propagates along the coaxial transmission line to the parallel detection ring probes. Due to impedance mismatch between the probe and the transmission line, part of the signal is reflected. The incident and reflected high-frequency waves superimpose to form a standing wave along the transmission line, resulting in variations in voltage amplitude at different points. The differential voltage between points *a* and *b* is extracted through the detection circuit and amplified to obtain the output voltage (U_{out}) of the stem water content sensor (Xu *et al.*, 2023; Xu *et al.*, 2024):

$$U_{\text{out}} = \beta(V_a - V_b) = 2\beta A \left(\frac{Z_l - Z_0}{Z_l + Z_0} \right) \quad (1)$$

In the equation, β represents amplifier gain coefficient; A is the amplitude of the high-frequency signal source; V_a and V_b denote the voltages at the two ends of the coaxial transmission line, as measured by the detection circuit. Z_0 denotes the impedance of the transmission line, which is 50Ω ; Z_l is the impedance of the detection ring. Given that A , β and Z_0 are constants, the U_{out} is only determined by the Z_l , which is directly related to its dielectric constant (Zhou *et al.*, 2018; Tian *et al.*, 2022).

Previous studies have demonstrated that the dielectric constant of liquid water is far greater than that of stem dry matter or solid ice, changes in stem water content (including tissue hydration/dehydration and freeze–thaw transitions of stem water) alter the dielectric constant, thereby changing stem impedance and ultimately causing a shift in the sensor's output voltage (Sun *et al.*, 2019; Xu *et al.*, 2024). Multiple preliminary experiments conducted by our team confirmed a strong linear correlation between sensor output voltage and stem water content. In addition, environmental factors such as ambient temperature, humidity, and electrical conductivity exerted only minimal disturbance on sensor performance, indicating that the system is relatively stable (Tian *et al.*, 2022; Xu *et al.*, 2023).

It should be noted that this study primarily focused on analyzing variations in stem water during the monitoring period rather than determining its absolute values. Therefore, the change in output voltage of the stem water content sensor was directly used as an indicator of internal stem water dynamics, hereafter referred to as the stem water index (Xu *et al.*, 2023; Xu *et al.*, 2024).

Before applying the different cold-resistance treatments, each stem water content sensor was installed on the *Malus spectabilis* stem at a height of approximately 20 cm above the ground, where the average stem diameter was about 6 cm.

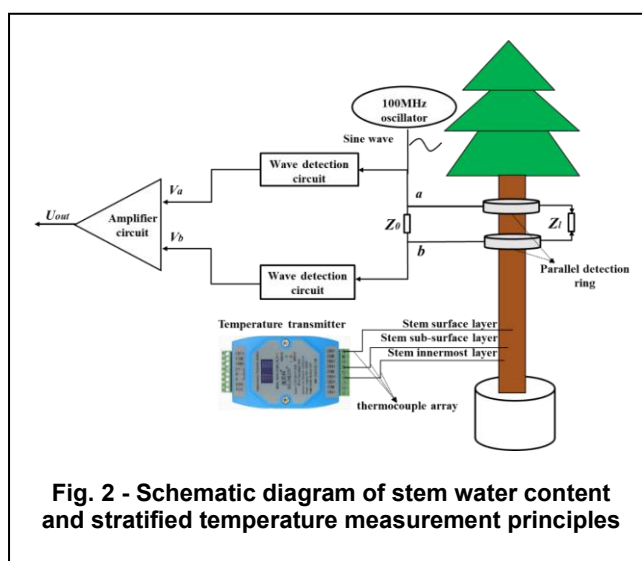
Measurement of radially stratified stem temperature

In perennial woody plants, stems are composed of multiple tissues formed during growth, including bark, cambium, and xylem (sapwood and heartwood) (Tulik *et al.*, 2019). These layered stem tissues differ in water content, and their temperatures also vary depending on depth beneath the bark surface. Therefore, in this study, the temperature profiles of stratified stem tissues were monitored.

Radial temperature profiles within the stem were obtained using a miniature thermocouple array (type K, 0.5 mm in diameter) with a measurement range of -200 to 1300°C . This type of thermocouple features good linearity, high sensitivity, and reliable stability and uniformity, making it suitable for field monitoring of stem temperature. The temperature data were recorded using an RS20K-C temperature transmitter (Sulinke, Guangdong, China).

The transmitter operates under the RS-485 communication protocol and is equipped with eight electrically isolated input channels, providing strong anti-interference capability and effectively preventing signal crosstalk. Cold-junction compensation for the thermocouples was achieved using a negative temperature coefficient (NTC) sensor integrated within the transmitter.

The installation procedure was as follows: three miniature thermocouples were installed in the stem of each *Malus spectabilis* sample. The array was mounted at a height of 10 cm above the ground, where the average stem diameter was approximately 6 cm. The insertion depths were set to 0 cm, 1 cm, and 2 cm, corresponding to the stem surface layer, sub-surface layer, and innermost layer, respectively. Small pilot holes (0.5–1 mm in diameter) were drilled evenly into the stem using a handheld drill. Each thermocouple was carefully inserted until the sensing tip reached the designated depth. Although this method leaves tiny holes on the stem surface and inside the tissues, the impact on the normal physiological activities of living trees is negligible, thus meeting the requirements of non-destructive monitoring (Xu *et al.*, 2021). During the monitoring process, the acquisition interval for radially stratified stem temperature was set to 10 minutes, consistent with that of stem water measurements. The schematic diagram of the thermocouple sensor is shown in Figure 2.



Data acquisition and monitoring system establishment

In this study, each stem water content sensor and the RS-485 temperature transmitter were connected to a self-developed multi-channel data acquisition unit (model ZRDL1001) based on an ATMEGA2560 microcontroller. The sensors were connected respectively to the analog-to-digital (AD) and RS-485 communication channels of the data acquisition unit. Data from all sensors were automatically recorded at 10-minute intervals and stored in the internal memory module (SD card) of the collector.

To enable real-time online monitoring and system status verification during the observation period, a 4G DTU module equipped with a SIM card was connected to the collector via the RS-232 interface. Using GPRS communication technology, the collected and packaged sensor data were transmitted to a remote Alibaba Cloud server. A self-developed intelligent forestry ecological monitoring platform was then used to visualize and download the transmitted data in real time.

Through this configuration, a complete Internet of Things (IoT)-based plant freeze–thaw monitoring system was established. Continuous monitoring of stem water content and radially stratified stem temperature in *Malus spectabilis* began in September 2021.

Freeze–thaw calculation model

In this study, the acquired radial stem temperature profiles and stem water content data were incorporated into the development of a stem freeze-thaw computational model, enabling the detection and analysis of stem ice content during the freeze-thaw process.

During the overwintering period, the intensity of physiological activities in trees gradually declines. Simultaneously, canopy leaves are shed, and both photosynthesis and transpiration decrease to relatively low levels, resulting in the lowest transpiration rates of the year. At this stage, freeze-thaw cycles occur within the stem tissues. The total water content of the stem (V_{stem}), including both ice and liquid water, remains relatively stable, while the stem volumetric ice content (V_{ice}) and stem liquid water content (V_{water}) approach thermodynamic equilibrium, such that their sum can be considered approximately constant (Ishikawa et al., 2009; Sun et al., 2019).

A schematic diagram of the stem cross-section is shown in Fig. 3. The red points represent the thermocouple array installed as described in the previous section, corresponding to measurement points 0, 1, and 2. Measurement point 0 approximates the ambient air temperature. Previous studies have demonstrated that the freezing and thawing processes within stem tissues proceed radially from the outer to the inner layers (Bozonnet et al., 2024). Building upon this finding, the freeze-thaw dynamics during the overwintering period were described as follows:

Freezing process: When the temperature at measurement point 1 reaches the freezing point, layer L0 is assumed to be fully frozen. When the temperature at measurement point 2 reaches the freezing point, layer L1 is considered fully frozen. Furthermore, as the temperature at point 2 continues to decrease to its minimum value, the volumetric fraction of ice in the stem is assumed to reach 1, indicating that all liquid water within the stem tissues has been converted into ice nuclei.

Thawing process: When the temperature at measurement point 1 rises to the freezing point, layer L0 is assumed to be completely thawed. When the temperature at measurement point 2 reaches the freezing point, layer L1 is considered fully thawed. Similarly, as the temperature at point 2 continues to increase to its maximum value, the volumetric fraction of ice in the stem is assumed to decrease to 0, indicating that all ice nuclei within the stem tissues have melted into liquid water.

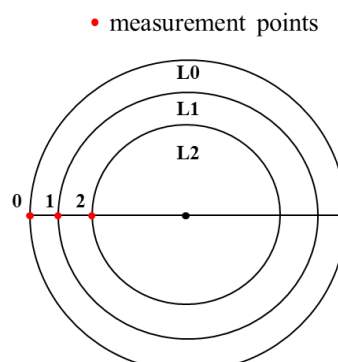


Fig. 3 - Schematic diagram of a stem cross-section

Define D_{freeze} , R and H as the frozen thickness of stem tissue, the thawed thickness of stem tissue, the stem radius, and the stem height, respectively (Fig. 4). Based on these parameters, a freeze-thaw calculation model for the stem was constructed.



a) Ice nucleation spreading from outer to inner layers b) Ice nucleation melting from outer to inner layers

Fig. 4 - Schematic diagram of freeze-thaw in stem tissues

The ice content in the stem volume (represented by η) is determined based on the definition of volume fraction:

$$\eta = \frac{V_{stem} - V_{water}}{V_{stem}} = \frac{V_{ice}}{V_{stem}} \quad (2)$$

When the temperature drops below freezing, the liquid water in the stem tissue begins to turn into solid ice, accompanied by the radial diffusion of ice nuclei from the outside to the inside, gradually forming a freezing peak inward. The volume ice content (η_{freeze}) during the freezing process is calculated as follows based on D_{freeze} , R and H :

$$\eta_{freeze} = \frac{V_{ice}}{V_{stem}} = \frac{\pi R^2 H - \pi (R - D_{freeze})^2 H}{\pi R^2 H} \quad (3)$$

The above equation can be simplified to yield the following expression:

$$\eta_{freeze} = \frac{R^2 - (R - D_{freeze})^2}{R^2} \quad (4)$$

From the above formula, it can be inferred that when all liquid water within the stem tissues has frozen, η_{freeze} increases from 0 to 1 as it D_{freeze} gradually rises from 0 to approach R .

Similarly, when the temperature gradually rises above the freezing point, the ice nuclei on the outer layer of the stem tissue melt radially from the outside inward, starting to form a melting peak; At D'_{freeze} this point, the D_{freeze} maximum freezing thickness (which is constant during the melting process, i.e., the stem radius R) is reached. The D'_{freeze} volume ice content during the melting process is also calculated based on D_{thaw} , R , H and D'_{freeze} :

$$\eta_{thaw} = \frac{V_{ice}}{V_{stem}} = \frac{\pi (R - D_{thaw})^2 H - \pi (R - D'_{freeze})^2 H}{\pi R^2 H} \quad (5)$$

The above equation can be simplified to yield the following expression:

$$\eta_{thaw} = \frac{(D'_{freeze} - D_{thaw})(2R - D'_{freeze} - D_{thaw})}{R^2} \quad (6)$$

From the above equation, it can be inferred that as the stem tissues transition from a fully frozen state to a completely thawed (liquid water) state, D_{thaw} gradually increases from 0 to approach R , while η_{thaw} decreases from 1 to 0.

During the freezing-thawing process of the stem, when $D_{freeze} = D_{thaw}$, the following relationship can be expressed:

$$\eta_{freeze} + \eta_{thaw} + \left(\frac{R - D'_{freeze}}{R}\right)^2 = 1 \quad (7)$$

RESULTS AND DISCUSSION

Analysis of radially stratified stem temperature and water content indicators

Prior to analyzing the freeze-thaw status of *Malus spectabilis*, this study examined the stratified stem temperature data and stem water content indicators obtained during the monitoring period from September 1, 2021, to April 12, 2022.

Figure 5 illustrates the variation in radially stratified stem temperature of *Malus spectabilis* during the overwintering period. It can be observed that the temperature profiles of different layers within the same stem exhibit similar trends, fluctuating around the freezing point, with the proportion of temperatures below 0 °C increasing markedly over time. As the bark serves as a boundary layer of the tree, it not only captures solar radiation but also provides excellent thermal insulation (*Webster et al., 2016; Tulik et al., 2019*), resulting in stem temperatures that are often higher than the ambient air temperature (*Zhao et al., 2021*). Consequently, it can be seen from the figure that, at any given moment during the monitoring period, the innermost layer exhibited the highest temperature, followed by the middle layer, while the outermost layer showed the lowest. Previous studies have reported that the stem surface temperature responds almost instantaneously to fluctuations in ambient air temperature, whereas temperature changes within deeper stem tissues occur with a delay of several minutes to several hours (*Tsalagkas et al., 2019*). The experimental results presented in Figure 5 partially confirm this conclusion.

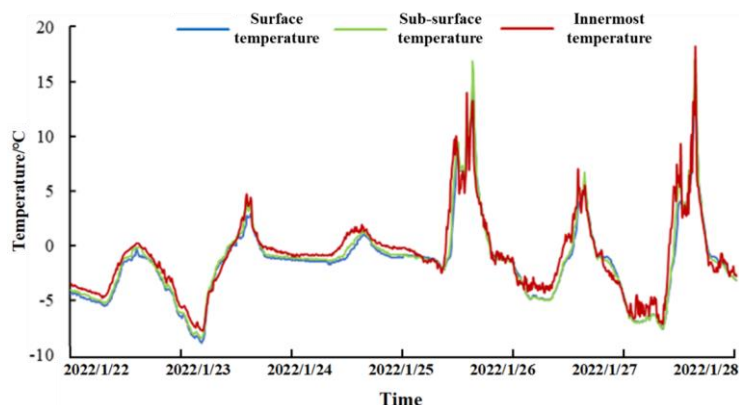


Fig. 5 - Variation in radially stratified stem temperature of *Malus spectabilis* during the overwintering period

Subsequently, the surface stem temperature variations of different *Malus* samples were compared to evaluate the effectiveness of the stem-wrapping frost protection measures (Figure 6). During the overwintering period, the intensity of physiological and metabolic activities in plants is greatly reduced, resulting in limited endogenous heat generation, and thus the heat of trees originates almost entirely from solar radiation. Under direct sunlight during the daytime, stem temperature gradually increases, whereas at night, as the intensity of photosynthetically active radiation diminishes, the plant can no longer acquire energy and the stem temperature drops sharply. Such drastic diurnal temperature fluctuations may cause bark cracking.

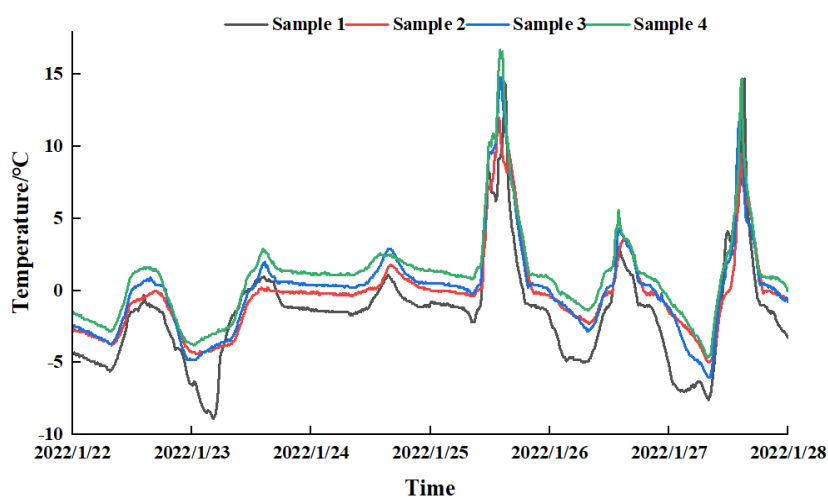


Fig. 6 - Comparative curves of surface temperature among different *Malus spectabilis* samples

When frost protection measures are applied, namely wrapping the stem with plant fiber materials, the intensity of direct solar radiation during the day is reduced, and the dissipation of heat at night is moderated. This indicates that wrapping treatment can buffer temperature fluctuations.

Accordingly, Sample 1 exhibited the largest diurnal variation in surface stem temperature, while the other samples showed smaller fluctuations. Moreover, Sample 1 experienced the longest duration of stem temperature below 0°C, whereas Sample 4 exhibited the shortest duration. These results suggest that implementing frost protection measures during the overwintering period can protect trees from cold damage, delay the onset of stem ‘freezing’ or accelerate the process of stem ‘thawing’, thereby reducing the time that stem temperature remains below the freezing point, moderating temperature fluctuations, and lowering the risk of bark cracking.

The freeze–thaw status of trees is not only associated with stem temperature but is also directly related to changes in internal stem water indicators. Figure 7 shows the variation curve of the stem water indicator for Sample 1 of *Malus* during the entire non-growing season. Overall, from early November to early December 2021, as the plant transitioned from the leaf-fall stage to the overwintering dormancy stage, the gradual decline in ambient temperature significantly weakened photosynthesis, transpiration, and root activity. Consequently, the amount of water charging and discharging in the stem tissue began to decrease, leading to a gradual downward trend in the stem water indicator (*Beedlow et al., 2017; Xu et al., 2024*).

From early December 2021, as the plant entered the overwintering dormancy stage, the continuous decrease in temperature caused the liquid water inside the stem tissue to begin freezing. At this stage, the stem water indicator sharply decreased to lower values. When the temperature gradually increased, the solid ice within the stem tissue melted back into liquid water, and the stem water indicator rose to higher values. Thus, it can be observed that the amplitude of stem water fluctuations increased significantly during this period. The alternating freeze–thaw phenomenon in stem tissue persisted until the end of February 2022. Previous studies have suggested that during this stage, stems and other tissues contain small amounts of sugars or electrolytes at relatively high concentrations, acting as antifreeze agents to lower the freezing point of the tissues and thereby largely reducing damage to cells caused by water expansion during freezing (*Goswami et al., 2022*).

From March 2022, as the plant transitioned from overwintering dormancy to the budding stage, both ambient and soil temperatures continued to rise, and plant vitality gradually increased (*Kumar et al., 2022*). The solid ice content within the stem tissue decreased, indicating the gradual disappearance of freeze–thaw processes. The fluctuation range of the stem water indicator became markedly reduced compared with the earlier stage and then gradually increased to higher levels similar to those observed during the leaf-fall stage (*Xu et al., 2024*). The stem water variation characteristics of *Malus* observed in this study during the entire non-growing season showed certain differences from those reported by Xu and Zhao in *Acer truncatum*, *Lagerstroemia indica*, and *Populus* stems (*Zhao et al., 2021; Xu et al., 2024*), suggesting that different tree species exhibit varying adaptive capacities to freeze–thaw stress during overwintering (*Li et al., 2023*).

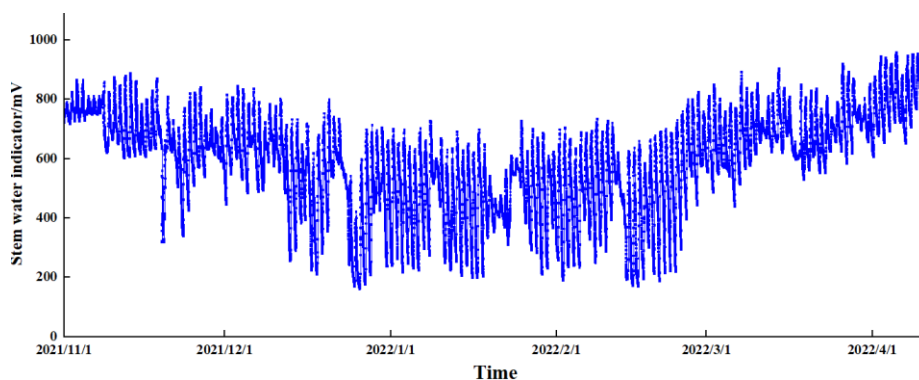


Fig. 7 - Variation curves of stem water content indicators for *Malus spectabilis* sample 1 during the entire dormant period

Meanwhile, during the overwintering period, the soil undergoes repeated freezing and thawing, leading to a gradual decline in root activity. As a result, the absorption of soil water—an essential source for sustaining normal plant physiological functions—decreases significantly (*Ambroise et al., 2020*), and

the liquid water content within stem tissues also declines. At the same time, the cold and dry northern winter winds increase plant transpiration and water loss. The combined effects of these factors may ultimately cause tree mortality due to water deficit during the overwintering stage.

To address this, the present study further evaluated whether the different types of frost protection measures employed could mitigate water loss. The results are shown in Figure 8. It can be observed that at the same time points, Sample 4 exhibited the highest stem water indicator values, while Sample 1 showed the lowest. This may be attributed to the fact that Sample 1 received no frost protection treatment, leaving the stem tissues fully exposed to the environment, thereby increasing transpiration-driven water consumption and resulting in significantly lower stem water values compared with the other samples. These findings indicate that wrapping tree stems with plant fiber materials not only provides thermal insulation and frost protection but also contributes to reducing stem water loss to a certain extent.

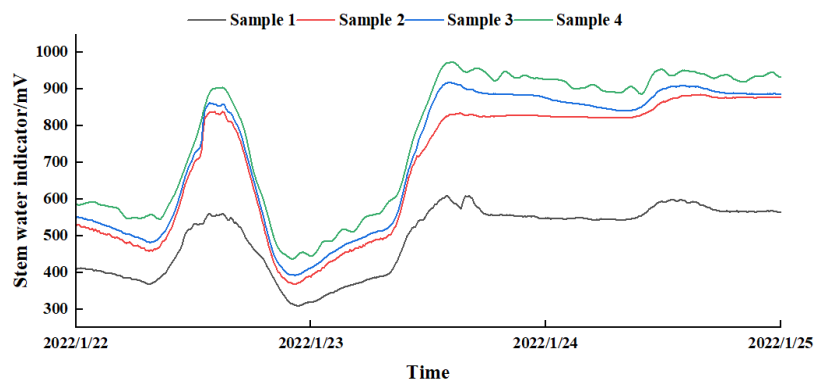


Fig. 8 - Comparison of stem water-related parameters in *Malus spectabilis* samples with different cold-protection treatments during the overwintering period

Detection of freeze-thaw status in *Malus spectabilis*

Based on the above analyses, a preliminary understanding of the cold resistance characteristics and regulatory mechanisms of the plant was obtained. Subsequently, representative curves of stem stratified temperature and water-related indicators were selected for detailed analysis (Figure 9). It was observed that during the overwintering period, both the stem water indicator and the surface stem temperature of *Malus* generally exhibited single-peak and single-trough periodic variations, i.e., a diurnal pattern of “rising during the day and falling at night.” In most cases, as temperature decreased, the surface, sub-surface, and innermost stem temperatures of the same sample declined sequentially; conversely, as temperature increased, the three layers rose in the same order. This suggests that ice nuclei within stem tissues diffuse radially from the exterior to the interior during freezing, and that the thawing of frozen regions also proceeds from the exterior inward, which is consistent with the theoretical framework of the freeze–thaw calculation model proposed earlier.

Previous studies have indicated that, due to the solute and matrix properties of stem tissues, the freezing point of solutes in apple (*Malus domestica*) stems is approximately -4°C (Sun *et al.*, 2019). As *Malus spectabilis* and apple (*Malus domestica*) both belong to the Rosaceae family, the two species share similar physiological characteristics (Zhang *et al.*, 2023). Figure 9 presents the stratified stem temperature and water indicator curves of Sample 1 during the overwintering period. The results show that starting at 15:20 on January 22, as stem temperature gradually declined to approximately -4°C , i.e., the stem freezing point, freezing within the tissues began. At this point, the rate of temperature decrease slowed noticeably. With further temperature decline, stem water gradually froze into ice nuclei, decreasing continuously until stabilizing at a low level. Conversely, as the stem temperature rose to approximately -4°C , the ice nuclei within stem tissues began to melt, and the rate of temperature increase likewise exhibited a gradual slowing. During this stage, the stem water indicator first increased slowly, then rose rapidly to a higher level.

Notably, during both freezing and thawing, the stem temperature curves displayed relatively stable phases concurrent with changes in stem water, reflecting energy exchange during the phase transition between liquid water and solid ice. In other words, freezing was accompanied by heat release, while thawing was accompanied by heat absorption (Gao *et al.*, 2021). This observation is consistent with the latent heat phenomena reported by Zhao and Tian in their studies of tree freeze–thaw

processes (Zhao et al., 2021; Tian et al., 2023).

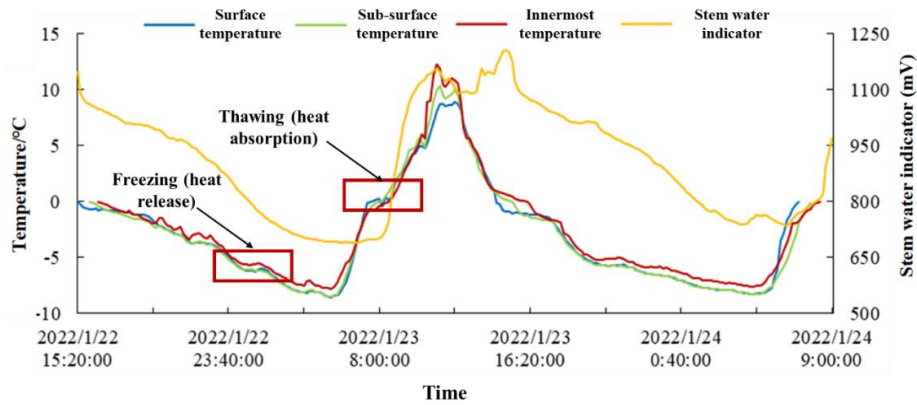


Fig. 9 - Variations in radially stratified stem temperature and stem water content of Malus spectabilis sample 1 during the overwintering period

The freeze–thaw status of Malus was further analyzed in conjunction with the freeze–thaw calculation model. The miniature thermocouple arrays installed on each tree allowed monitoring of temperature at the surface layer (0 cm), sub-surface layer (1 cm), and innermost layer (2 cm) of stem tissues with an average radius of approximately 3 cm. According to the freeze-thaw calculation model, when the temperature at the innermost layer reaches its minimum, all liquid water within the stem tissue is assumed to be completely frozen, that is, that is, $D_{freeze}=R$, indicating that the volumetric ice content of the stem (η) equals 1. Conversely, when the innermost layer temperature reaches its maximum, all solid ice within the stem tissue is assumed to have melted into liquid water, that is, $D_{thaw}=R$, indicating that $\eta = 0$.

Since the thermocouple arrays can only provide temperature data at specific depths and cannot capture continuous temperature variations throughout the stem, this study first calculated the volumetric ice content at these discrete positions. Subsequently, the freezing and thawing processes of stem volumetric ice content were fitted in segments. The fitting equation is as follows:

$$y = y_0 + \frac{A}{\sqrt{2\pi}wx} e^{-\frac{\left(\ln\frac{x}{xc}\right)^2}{2w^2}} \quad (8)$$

The fitted curves of stem volumetric ice content during the freezing and thawing processes are presented in Figure 10.

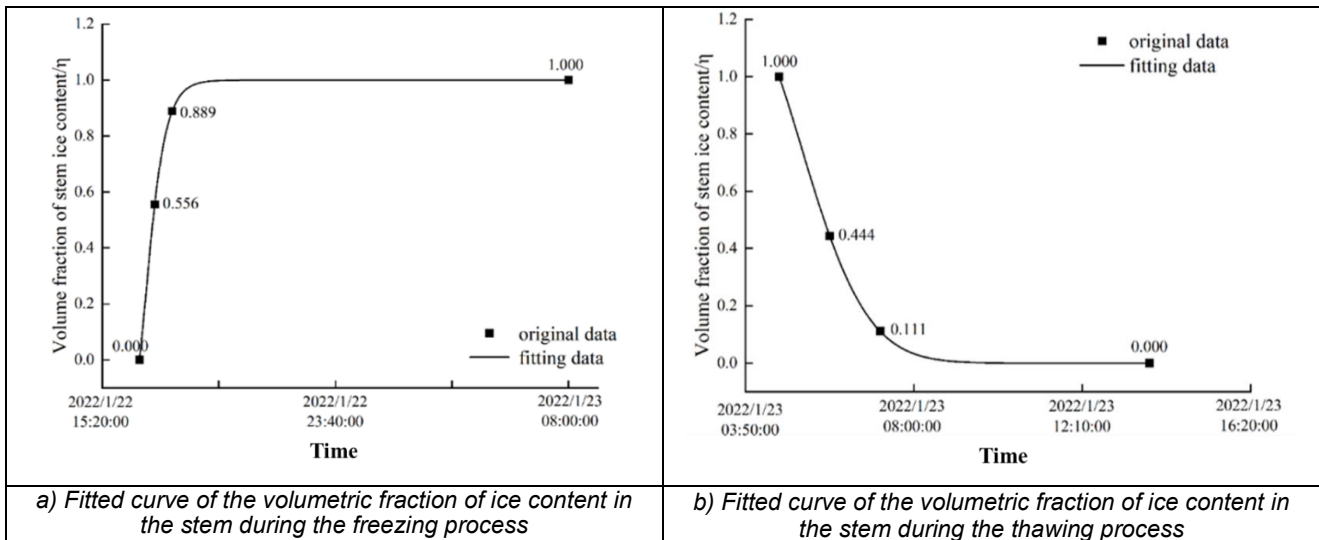


Fig. 10 - Fitted curves of the volumetric fraction of ice content in the stem during the freezing and thawing processes

Figure 11 presents the variations in radially stratified stem temperature and the fitted curves of the volumetric ice fraction (η) in *Malus spectabilis* samples during the freeze–thaw process. It can be observed that η exhibits an opposite trend to the stratified stem temperature. During the cooling phase, as η increases from 0 to 1, ice nuclei within the stem diffuse radially from the exterior toward the interior, and the freezing peak migrates inward.

This indicates that the freezing thickness D_{freeze} increases from 0 to R , meaning that the radial freezing depth of the stem continuously expands. Conversely, during the warming phase, as η decreases from 1 to 0, the frozen regions melt progressively from the exterior toward the interior, and the thawing peak also migrates inward. This indicates that the thawing thickness increases D_{thaw} increases from 0 to R , meaning that the radial thawing depth of the stem continuously expands.

These observations demonstrate that freezing and thawing within stem tissues proceed radially from the outer toward the inner layers, with such processes occurring earlier in tissues closer to the surface (Tian *et al.*, 2020; Zhao *et al.*, 2021). In summary, by tracking the dynamic variations in freezing and thawing thickness within stem tissues, the migration trajectory of the freeze–thaw peaks can be effectively monitored, and this trajectory exhibits a distinct periodicity.

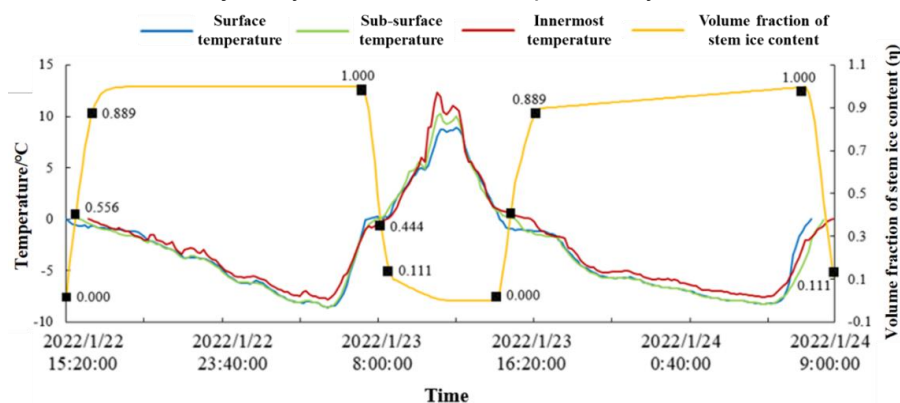


Fig. 11 - Variations in stem temperature and volumetric ice content of *Malus spectabilis* sample 1 during the overwintering period

CONCLUSIONS

This study focuses on common ornamental tree species in northern China during the wintering period and evaluates the effectiveness of various cold-protection measures applied to the tree trunks. Simultaneously, a plant freeze–thaw monitoring system was established, employing an independently designed stem water content sensor based on the standing wave ratio principle in combination with a micro-thermocouple array. This system enabled the acquisition of stem water content and radially stratified temperature data during the wintering period, and the dynamic characteristics of these parameters were analyzed. Based on these observations, a freeze–thaw computational model was constructed. The main contributions of this study include:

(1) It is effective to use plant fiber materials to protect trees from cold, and it can reduce the loss of water from the stems of trees;

(2) The stem water content and stem layer temperature information basically showed synchronous changes. The stem water content showed a gradual downward trend from the leaf fall period to the freezing and thawing dormant period. During the overwintering period, the stem temperature continued to decrease, causing the stem water content to begin to alternate between freezing and thawing, accompanied by heat absorption and release. The fluctuation range continued to increase and dropped to a lower level, which continued until the budding period. Subsequently, the water content in the stem began to gradually increase.

The freeze-thaw calculation model indicates that the freezing and thawing processes within the stem tissue progress radially from the epidermis inward, implying that the freeze-thaw peaks migrate from the outside inward, indicating an increasing freeze-thaw thickness. During this process, the freezing of the stem is accompanied by a change in ice content η from 0 to 1, while the melting of the stem is accompanied by a change in ice content from 1 to 0.

This study can provide a novel perspective for the research on freeze-thaw detection of forest trees, and can provide a theoretical basis for the scientific formulation of cold-proof measures for forest trees during the winter period.

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