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Thermographic analysis of leaf water and energy information of Japanese spindle and glossy privet trees in low temperature environment☆

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ABSTRACT

Thermography has been used in many fields to perform non-invasive temperature measurements of natural objects. In this paper, thermography was used to determine the temperature of leaves, stems and branch kerfs of Japanese spindle (*Euonymus japonicus* Thunb.) and glossy privet (*Ligustrum lucidum* Ait.) in the city of Jinan in China during winter. The temperatures of the leaves, stems and branch kerfs were monitored as the temperature decreased after the sample was subjected to hand heating or after the branch was cut. Differences in the specific heats and the latent heats of the leaves, branches and stems with different water contents and transpiration capacities were confirmed. The significant temperature difference obtained after hand heating between different leaf sections with varied water contents made it easy to obtain the thermal images, which were clear and exhibited reduced systematic errors. After hand heating, a significantly higher temperature was found at the major vein system of both Japanese spindle and glossy privet. This increased temperature difference made it possible to detect the water and the thermal state of these leaves. Therefore, it was possible to detect scorched area of the leaves, the twig dieback and the sap warming phenomenon in the leaves using thermography. In addition, the leaf bending phenomenon observed in Japanese spindle leaves during the deep freezing process indicates that the leaf scorch symptoms result from water stress and a lack of sap warming.

1. Introduction

The specific heat and the latent heat of the melting and evaporating process of water lay the foundation of transpiration cooling (Clements, 1934; Rosenberg, 1974). With the highest specific heat, water tends to stabilize temperature, and this process is reflected in the relatively uniform temperature of islands and lands near a large body of water (Kramer, 1983; Rosenberg, 1974). Transpiration causes the temperature of trees to vary less than the air temperature (Gates, 1968). The lower temperature of deeper soil water and soil water under the coverage of crown self increases the effectiveness of the transpiration cooling of trees (Rosenberg, 1974). The soil temperature amplitude decreases with increasing depth during both summer and winter. At a depth of 40 cm, the temperature wave is significantly damped, particularly in winter, whereas no significant diurnal/annual wave is found at a depth of 80 cm (Rosenberg, 1974). In Jinan, the maximum difference between the air temperature and the deeper soil temperature reach more than 10 °C in the summer and more than 5 °C in the winter. Therefore, the water under a deep layer of soil results in the slight variation observed in the temperature of trees compared with the air temperature.

However, plant transpiration has been considered an unavoidable evil for a long period of time (Kramer, 1983). The active action of water in the transpiration cooling of plants has even been disregarded to some extent, and the warming action of sap water in winter has not been analyzed. During the winter, the warm sap from deep soil plays an important role in the cold hardness of many plants, especially in Jinan City, China. In this paper, we evidenced the "sap warming" process of some evergreen tree/shrub species during a cold Jinan winter using thermography. The proper consistency between the leaf tip and margin scorched areas and the lower temperatures in the same area suggest that this type of symptom results from a lack of warm water from the root system.

Various noises and small temperature differences in field measurements make it difficult to compare different thermal images (Chaerle and Van-Der-Straeten, 2000; Chaerle et al., 1999; Grant et al., 2006). To increase the comparability of this type of images, some researchers have attempted to use contrast models (Jones and Leinonen, 2003) in thermographic detection. Nilsson (1995) observed a significant leaf temperature decrease in a gust of wind, which implies that the dynamics of the imaging temperature are important for the identification of the stress status of plants. In this study, hand-heated leaves were

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used to study the leaf temperature and the sap warming phenomenon in a winter environment. The significant temperature difference on the heated leaf made it easy to obtain the thermal image and reduced the systematical error. In this study, the temperatures of the leaves and twigs of Japanese spindle and glossy privet were detected using thermography during the cooling process after hand heating. The significantly amplified variation in the imaging temperature between the normal and severed parts of leaves and branches during the cooling process after hand heating indicated that it is possible to detect the freezing dehydration of leaves, the scorching of leaves and the branch dieback by analyzing their changes using thermography. Moreover, these freezing stresses from lack of sap warm can be early detected before the appearance of any visible symptoms.

2. Materials and methods

2.1. Background of the study

This study was performed from December 2012 to January 2013 near the Yanshan crossroads in Jinan City, China, which is located at E 117º 0′ 0″ and N 36º 24′ 0″. This region has an extreme minimum temperature in January of −14.5 °C. During the study, the maximum and minimum temperatures were 8 and −13 °C, respectively. Three rounds of snowfall occurred on the days of Dec. 13–14, 20–21 and 28 in 2012, which resulted in 12.0, 9.6 and 7.0 mm, respectively, of precipitation. In December of 2012, the total precipitation was 29.2 mm, being the second highest amount of monthly precipitation in the years from 1951 to 2012. Therefore, it can be stated that the studied winter was a durative freezing winter. During this period, several green hedge stocks and roadside trees of Japanese spindle (*Euonymus japonicus* Thunb.) and glossy privet (*Ligustrum lucidum* Ait.) were studied. Some Japanese spindle specimens under the bridge of the Yanshan crossroads, where almost no water of rainfall or snowfall can be received, were studied, and compared with the normal specimens in the field.

2.2. Imaging temperature measured by using thermography

The temperatures of leaves, stems and branch kerfs were determined by using thermography technology (Jones, 1999; Jones and Leinonen, 2003; Jones et al., 2002; Prytz et al., 2003) with a NEC H2640 thermal infrared (8–13 μm) camera with a temperature measuring scope that ranged from −40 to 500 °C and a minimum sensible temperature of 0.03 °C. Throughout the measurements, the camera was handheld approximately 50 cm above the objective leaves/branches at front-lighting and focused to clarify the image. Smoothly expanded leaves were selected to avoid the systematic errors that are obtained in the collection of thermal images. Thermal images were obtained during the processes of hand-heating and non-hand-heating in the LVT automatic sensitivity tracing mode. After the target leaf was clamped between two hands and heated for approximately 10 s, thermal images were continuously taken as the temperature decreased until a temperature that was nearly equal to the around environmental temperature. The clearest thermal image was used to analyze the image temperature. The imaging temperatures were determined using the InfReC Analyzer NS9500 software provided with the camera. The difference in the temperature (*DT*) obtained from the thermal images of each duplicate was the difference between the average temperature and the minimum temperature of the duplicate. This value was calculated using Eq. (1):

$$Dt_i = (T_i - T_{\min}) \tag{1}$$

where Dt_i is the difference in the temperature of the ith duplicate ($i =$ 1,2… m, where m is the number of duplicates). T_{min} is the minimum temperature value of the ith duplicate, and T_i is the average temperature value of the ith duplicate.

2.3. Analysis of RGB images

In the study, the leaves and stems were monitored during the process of winter freezing from December of 2012 to January of 2013 through making both thermal and RGB images. The RGB images were obtained with a CCD camera (Fuji SL 305). These images were stored as tiff files. No special constraint was used in the determination of the leaf scorch area percent (LSAP), with the exception of blurry images. The LSAP values, which are a proportion between the scorched area and total area of the leaves, were defined and calculated using the method described by Wang and Omasa (2012).

2.4. Definition and calculation of the leaf angle between the petiole and the tip

The leaf angle between the petiole and the tip (LAPT) of the target leaves is a side-glance bending angle between the leaf petiole and the leaf tip (Fig. 1), which was measured using the RGB images obtained with the CCD camera (Fuji SL 305). This value was measured using the angle analysis tool in the UTHSCSA Image Tool 3.00 software. The LAPT was directly measured if it was a sharp angle (Fig. 1a), indirectly determined according to its corresponding angle if it was an obtuse angle (Fig. 1b) and directly measured according to the point of the leaf tip if it was a reflex angle (Fig. 1c).

2.5. Leaf water content

The water content (WC) of glossy privet leaves was differentially measured to compare the WCs in the leaf tip and the base. During the study, scorched leaves were separated into two sections: tip and base. The water contents of these sections were measured using the

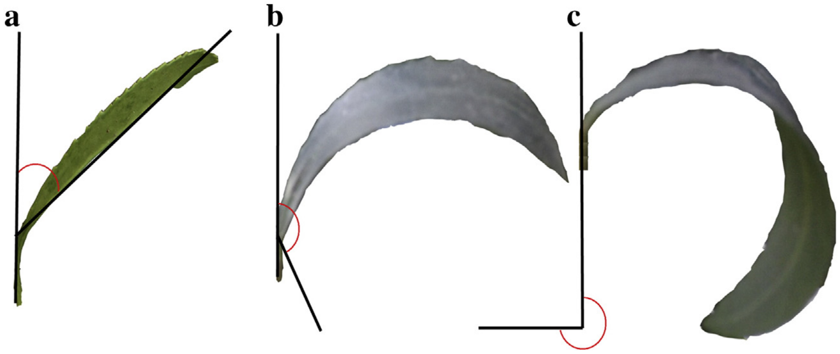

Fig. 1. Leaf angle between the petiole and the tip (LAPT): a. sharp angle, b. obtuse angle and c. reflex angle.

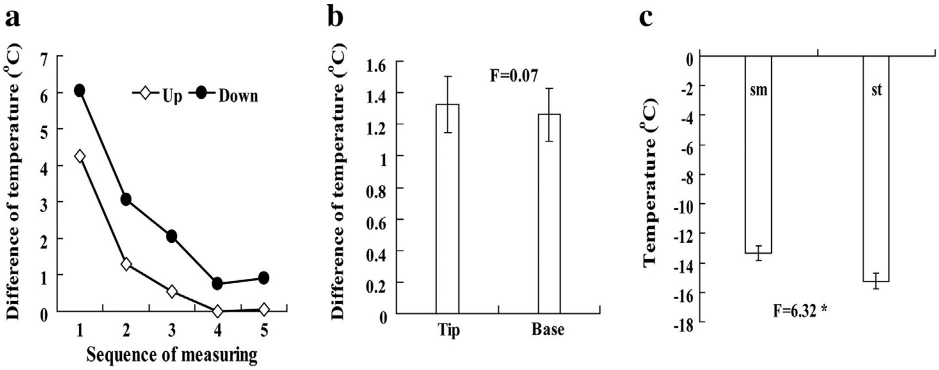

Fig. 2. Statistical results of leaf and branch kerf temperatures. a. Temporal series of temperature differences (mean-minimum) between upper branch kerfs (up) and lower branch kerfs (down); these temperature measurements show a lag characteristic of the decreasing temperature in the lower branches. b. Temperature difference (mean-minimum) between the tip and the base area of glossy privet leaves; the differences in the temperatures of the tips and bases are not statistically significant. c. Significant temperature difference between branch kerfs (sm) and main stems (st) of Japanese spindle seedlings.

rapid weighing method with a 1/10,000 g Shimadzu electronic balance (AUY 120) under room temperature conditions. The weight of the sampled leaf sections was weighed immediately after the sample was obtained from the field. After the dry weight was obtained, the water content was calculated using Eq. (2):

$$WC\% = \frac{FW - DW}{FW} \times 100 \qquad (2)$$

where WC is the water content, FW is the fresh weight of the samples and DW is the dry weight of the samples.

3. Results and analysis

3.1. Image temperatures in a normal winter environment

Every object continually exchanges energy with its environment and tends to maintain an energy balance. Plants attempt to always maintain their temperatures to equal to that of the surrounding environment; this is particularly true for the surface temperature after a persistent energy exchange (Fig. 2a). Therefore, the surface temperature of leaves (or different parts of the same leaf) usually does not significantly change in winter, especially at night and during cloudy days. All the measurements are not exhibiting statistically significant difference between the leaf tip and the base area (Fig. 2b). However, a higher temperature was occasionally found at the leaf base in the extreme cold environment, although the repeatability of this result was not high. In this study, the leaves of Japanese spindle and glossy privet were used as typical examples (Figs. 3a and b). The point temperature variance of the leaves usually ranged within 1.0 °C. However, during a sudden change in the environment, a significantly higher temperature was measured at the leaf base or the leaf venation system. This type of difference appeared to be the result of the high inner temperature of plant bodies. This hypothesis can be proven by the fact that the branch kerf of Japanese spindle (Fig. 3c–a) exhibited a higher instant temperature than the stem surface (Fig. 3c–b). This difference between the inner temperature and the surface temperature is often statistically significant (Fig. 2c), especially in an environment with an extremely low temperature and snow. The large temperature variance between the inner part of plants and the environment results in its easy detection by thermography. The sap that brings relatively warm water from underground to the terminal leaves maintains the persistent difference between the inner and the surface temperature. This finding is evidenced by the persistently higher kerf temperature in the lower parts of the stem (Fig. 2a-●-●) compared with the upper/terminal part (Fig. 2a-◊-◊) in Japanese spindle.

3.2. Thermatic analysis of leaf water and thermal status after hand heating

In normal winter environments, the temperature difference within a leaf blade is not easily measured (Fig. 4a), although a warmer leaf vein system is sometimes detected using thermography. The noise

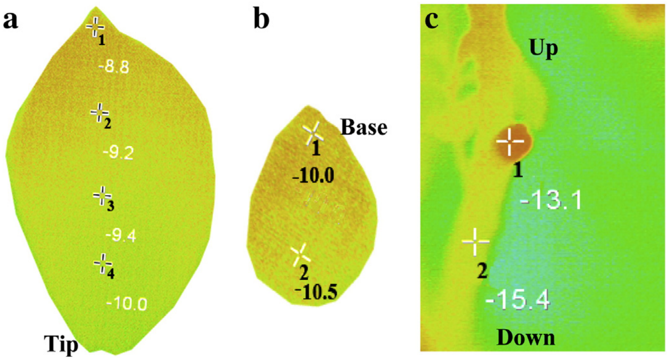

Fig. 3. Winter thermal images of the main stem and leaves of Japanese spindle and glossy privet. a. Thermal image of glossy privet leaf with gradient (high to low) temperatures from the base to the tip (a-1, a-2, a-3 and a-4). b. Thermal image of Japanese spindle with temperature (high to low) gradient from the base to the tip (b-1, b-2). The centers of the crosses indicate the point at which the temperature was measured. c. Main stem of a Japanese spindle seedling with high-temperature branch kerfs (c-1) and a low-temperature stem (c-2).

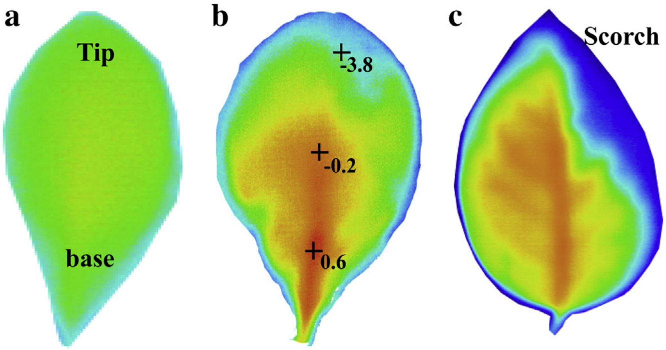

Fig. 4. Thermal images of Japanese spindle and glossy privet leaves as the temperature decreased after hand heating. a. Thermal image of a normal Japanese spindle leaf. b. Thermal image of a Japanese spindle leaf before the appearance of leaf scorch symptoms. c. Thermal image of a glossy privet leaf after the appearance of leaf scorch symptoms.

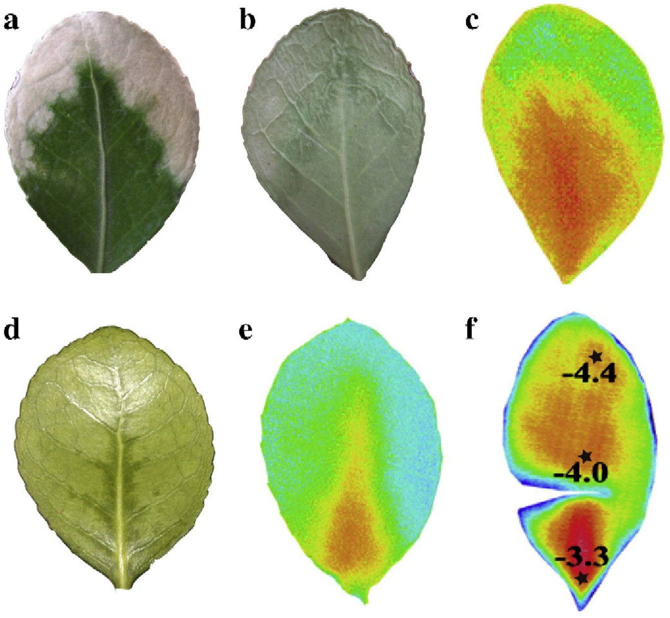

Fig. 5. RGB and thermal images of the front and back surfaces of a Japanese spindle leaf as the temperature decreased after hand heating. a. Front surface of a leaf with a clear tip and a margin scorch. b. Back surface of the leaf in (a) shows the thinner major or minor vein in the tip area. c. Thermal image of the leaf in (a) with almost the same low temperature at the tip and margin areas. d. A leaf with significant water trance at the base of the leaf and along the major vein. e. Thermal image of the leaf in (d) as the temperature decreased after hand heating. f. Thermal image of a Japanese spindle leaf, more than half of which was crosscut.

from the reflecting light of the leaf surface often resulted in a significantly large measurement error, particularly in the measurement of leaves with a bright luster, such as the surface of the Japanese spindle plant. To avoid this type of noise, we used the sunshine heating method to obtain thermal images with a significant temperature difference between different plant parts (Wang and Yamamoto, 2010) in the summer. In the present study, the hand-heating method was used in the thermal detection process. The temperature of the target leaves, which were heated by the observer's hand and thus subjected to a constant energy source of 36.8 °C, can reach approximately 20 °C. This heating resulted in clearer thermal images and a significant temperature difference between the different parts of leaves exposed to varied stress conditions. After hand heating, the temperature difference within a leaf can reach 3 to 4 °C (Fig. 4b), and 5–8 °C or more under extreme conditions, particularly in leaves with scorched tip and/or margin (Fig. 4c). Most of the thermal images obtained during the cooling process after hand heating showed a clear major vein system with a higher temperature (Figs. 4b and c) through the leaf, with the exception of a partial area with abruptly thinner veins. The thin leaf venation at the tip and margin of Japanese spindle leaves is a special example (Figs. 5a, b and c). In this special case, the higher temperature area is consistent with the area that presents a significant trace of water (Figs. 5d and e). This result demonstrated that the higher temperature of the major vein system is the result of its higher water content. This phenomenon can also be observed in the thermal imaging of a crosscut Japanese spindle leaf (more than half of the leaf is crosscut; Fig. 5f).

3.3. Identifying the leaf tip and margin scorches using thermal images

A statistically significant temperature difference between the scorched tip and the living base area of scorched leaves of Japanese spindle and glossy privet was measured after hand heating (Fig. 6a; $F = 5.43$ and $P < 0.05$). The scorched area of glossy privet measured using both thermal and RGB images of the same leaves showed no significant difference (Fig. 6b; $F = 0.002$ and $P > 0.1$). However, the typical temperature difference and temperature changes can be found between the living leaves and the wilted leaves of Japanese spindle after hand heating. Due to their low water content, the wilted leaves usually exhibit a higher temperature at the beginning of the measurement and a lower temperature at the end of the measurement compared with living leaves (Fig. 6c). After the scorched area is separated from the living area of glossy privet leaves, the scorched tip and margin areas exhibited a similar lower temperature and water content (Figs. 7c and d), whereas the living base area presented a higher temperature and a higher water content (Figs. 7c and d). Therefore, the hand-heating method can be potentially used to distinguish between dead and living stocks and between normal and scorched leaves of Japanese spindle and glossy privet.

The appearance of scorched symptoms in the tips and margins of the leaves of many tree species is related to their water metabolism (Wang and Omasa, 2012; Wang et al., 2009a, 2009b), particularly during periods of summer drought. Similarly, the scorched patterns observed in the tips and margins of Japanese spindle during the winter freezing dehydration process appear to be also related to a water imbalance. In this study, an evident leaf bend in the Japanese spindle was determined through the measurement of the LAPT (Fig. 7a; $F = 147.8$ and $P < 0.01$) during the freezing process. As the air temperature was raised to above 0 °C, the bended Japanese spindle leaves usually return to their normal turgid status (Fig. 7a) with a small LAPT. We also found a similar leaf bending phenomenon in detached Japanese spindle leaves in spring and measured a similar LAPT value (Fig. 7b). Moreover, more severe leaf tip and margin scorches were found in the leaves of Japanese spindle trees planted in dry and cement-polluted soil conditions compared with those found in the

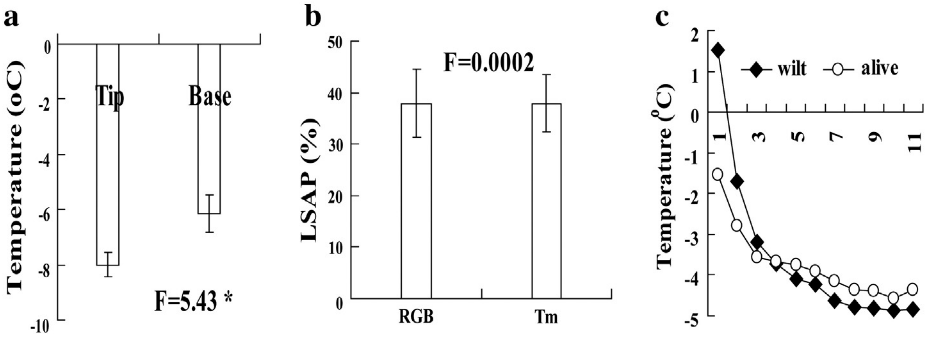

Fig. 6. Analysis of the thermal and RGB imaging of Japanese spindle. a. Temperatures of the tip and base leaf sections as the temperature decreased after hand heating. b. Comparison between the leaf scorch area percent (LSAP) obtained from the RGB images (RGB) with that obtained from the thermal images (Tm). c. Comparison of the temperature of living and wilted leaves of Japanese spindle.

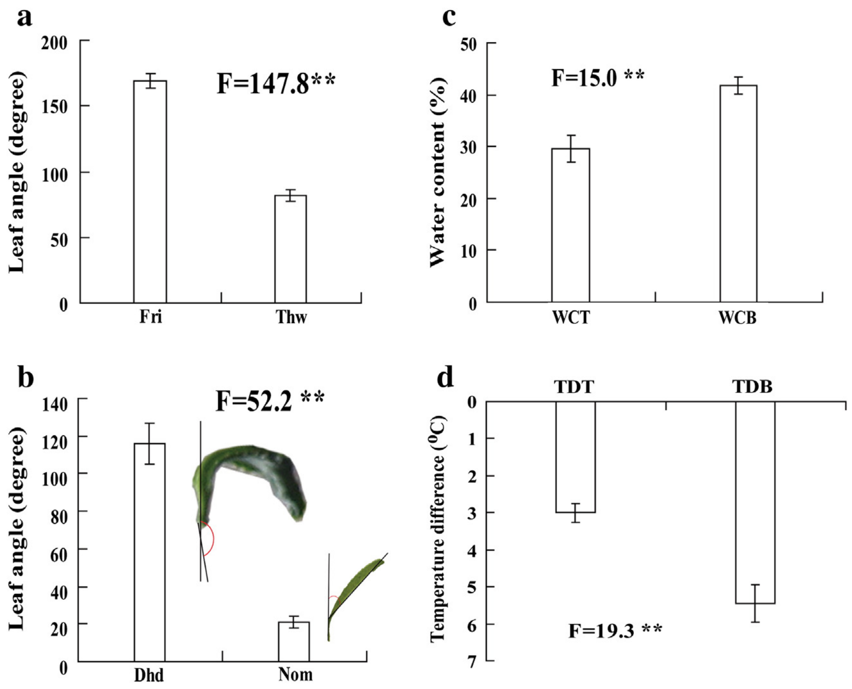

Fig. 7. Leaf angle of Japanese spindle and water content and temperature differences in glossy privet leaves. a. Leaf angle between the petiole and the tip of freezing-dehydrated Japanese spindle leaves (Fri) and thawing turgid leaves (Thw) in winter. b. Leaf angle between the petiole and the tip of air-dehydrated Japanese spindle leaves (Dhd) and normal turgid leaves (Nom) in spring. c. Water content of glossy privet leaf tip (WCT) and leaf base (WCB). d. Temperature difference between the tip (TDT) and the leaf base (TDB) of a glossy privet leaf.

normal conditions observed in Jinan City, China. Newly transplanted seedlings also exhibited the described scorch symptoms due to their imperfect root systems. Therefore, these findings suggest that the leaf tip and margin scorches found in Japanese spindle in the freezing conditions of winter are related to a water imbalance and/or a lack of sap warming.

4. Discussion

In the analysis of the damage to plants/trees that is caused by freezing, a long-debated question is why only the leaf tip and/or the branch top is hurt by the almost evenly ranged cold environment. This question has not been addressed by many previous research studies. Using imaging, we found that the structure of both the leaves and branches of Japanese spindle and glossy privet exhibits a heterogeneous property. The abruptly thinner major veins at the leaf tip and/or the margin area result in their lack of sap warming, which makes these plant parts sensitive to freezing damage. Inversely, the leaf and branch base, which are directly connected to the main stem/vascular system, tend to continuously receive warm sap from temperature-stabilized deep underground water.

Although we sometimes observed the warmer leaf venation system by thermography, a significantly higher temperature was found in the major vein in the thermal images obtained after hand heating. In addition, we found consistent correlations between areas with high water content and areas with a high temperature. This finding suggests that a lack of warm sap from underground causes Japanese spindle trees that are newly transplanted or planted in water-stressed environments to be sensitive to freezing damage. The heterogeneity of the major vein from the leaf base to the tip results in the leaf bend observed in Japanese spindle leaves during severe freezing dehydration (Levitt, 1972); this phenomenon is similar to the bending observed during the dehydration of detached leaves in spring. The huge tension in the water transport system easily snaps the water continuum at the thin part of the leaf during freezing dehydration, which obstructs the warm sap from the tip and margin area. Under water stress environments, the leaf tip and margin area are scorched due to a lack of sap warming. The leaf bending phenomenon observed in both freezing-dehydrated leaves and air-dehydrated leaves of Japanese spindle trees suggests that these processes have a similar mechanism. In addition, only a small number of Japanese spindle trees exhibit the leaf scorch symptoms in a normal environment due to their ability to maintain a high water content.

Hand heating effectively increased the temperature difference between the areas of the leaf with different water contents. As the temperature decreased from almost 20 °C to −15 °C, clearer thermal images were obtained with the thermal camera, which clearly show the snap point of the major vein. Therefore, because the different water contents of different leaf areas result in different specific heats, thermography is an effective tool that can be used to identify the different water and thermal states in leaves after hand heating. Thus, thermography can be potentially used to diagnose leaf scorch symptoms before the appearance of visible symptoms.

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