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Short communication

Study on the cause of peeling and withering in *Isodon rubescens* stems in winter

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ABSTRACT

Objectives: The living stems of *Isodon rubescens* (Hemsley) H. Hara peels and withers when the air temperature is below 0 °C in winter. The peels and withers evidently hamper the growth of *I. rubescens*. This report analyses the reason that causes *I. rubescens* stems to peel and wither in winter.
Methods: The field investigation and paraffin sections of *I. rubescens* stems (peeled and intact) were performed.
Results: The results showed that *I. rubescens* stems extruded ice ribbons at air temperature below 0 °C. The enlarged ice ribbons separated the phloem from the xylem, burst the phloem and even the whole bark of the *I. rubescens* stems.
Conclusions: The phloem was separated from the xylem by the enlarged ice ribbons. The phloem and the whole bark of the *I. rubescens* stems were torn when ice ribbons appeared. The damaged stem could not transport water and nutrition to the upper section. The upper stem above the ice ribbons withered because it could not receives water and nutrition.

1. Introduction

Isodon rubescens (Hemsley) H. Hara belongs to the Lamiaceae family (Flora of China Committee, 1979). *I. rubescens* is widely distributed in China (Flora of China Committee, 1979). The dry leaves and stems of *Isodon rubescens* are called rabdosiae rubescentis herba used in traditional Chinese medicine for the treatment of sore throat, inflammation, gastrointestinal problems, oesophageal cancer and so on (Chinese Pharmacopoeia Committee, 2020). *I. rubescens* is a kind of perennial subshrub. The root and rhizome of *I. rubescens* are woody and can grow for several years. The shoot tip of the *I. rubescens* stem is herbaceous and withers in winter. The middle and lower parts of *I. rubescens* stems are woody and able to sprout in spring, although their leaves fall in winter.

The upper part of the living stem of *I. rubescens* peeled and withered when the air temperature was below 0 °C in winter (Wang et al., 2008; Lu et al., 2007). The living stem of *I. rubescens* peeled and withered piece by piece if the air temperature fell below 0 °C several times. This was very unfavorable for *I. rubescens* growth. A field investigation and paraffin sectioning were performed to study the causes of peeling and withering in the living stem of *I. rubescens* in winter. This study can contribute to *I. rubescens* cultivation and resources protection.

2. Materials and methods

Instruments used were a camera (Nikon D5200, Nikon Group), a microtome (RM2125, Leica Microsystems Trading Co., Ltd) and a microscope (BA310, MoticMedical Diagnosis System Co., Ltd). All solvents, reagents and other chemicals (ethanol, dimethylbenzene, formalin, acetic acid, glycerol, paraffin, safranin and fast green) used were analytical grade.

The peeled *I. rubescens* stem and healthy (intact) one were collected from Jiulingshan Mountain in Henan province of China in January 2019.

The surface features and changes of *I. rubescens* stems were investigated at air temperatures below 0 °C on the Taihang Mountain in January 2019. The *I. rubescens* stems were photographed.

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The slide specimens of peeled and intact *I. rubescens* stems were prepared using the modified paraffin section method detailed below (Liu et al., 2016).

The specimens were placed in the fixation solution (90 ml of 70% (v/v) ethanol, 5 ml of acetic acid and 5 ml of formalin) for 24 h at first. Then the fixation solution was rinsed away, and the specimens were softened by soaking in solution containing 10 ml of glycerol and 10 ml of 95 % (v/v) ethanol for 2 h. The specimens were dehydrated in gradient solutions of 30 % (v/v), 50 %, 70 %, 85 %, 95 %, and 100 % ethanol for 2 h each time. The dehydrated specimens were successively soaked in the mixture of ethanol and dimethylbenzene (1:1, v/v) followed by dimethylbenzene (2 times) for 1 h each time to gradually clear. The cleared specimens were successively soaked in the mixture of dimethylbenzene and melted paraffin (1:1, v/v) followed by melted paraffin (2 times) for 1 h each time. Then the specimens were embedded in paraffin before sectioning. The embedded materials were sectioned (8–10 μm in thickness) on microtome. The sections were attached to a glass slide with gelatin and then baked in an oven at 40 °C for 5–10 min. The paraffin sections were successively soaked in dimethylbenzene (2 times), mixture of dimethylbenzene and ethanol (1:1, v/v) and then in ethanol (2 times) for 10 min each time. The dried paraffin sections were dyed with 0.3 % (w/v) fast green solution (dissolved in ethanol) for 1 min and then dyed with 0.5 % (w/v) safranin solution (dissolved in water) for 5 min. The dyed paraffin sections were sealed with gelatin and cover glasses.

The prepared paraffin sections were photographed under the microscope with a micro camera.

3. Results and discussion

Based on the field investigation, it was found that there were thin feathery ice ribbons on the ridge of living *I. rubescens* stems at air temperature below 0 °C. The ice ribbons burst the bark (Fig. 1). The detached barks appeared as longitudinal strips. The

Fig. 1. The burst bark on *I. rubescens* stem due to ice ribbons.

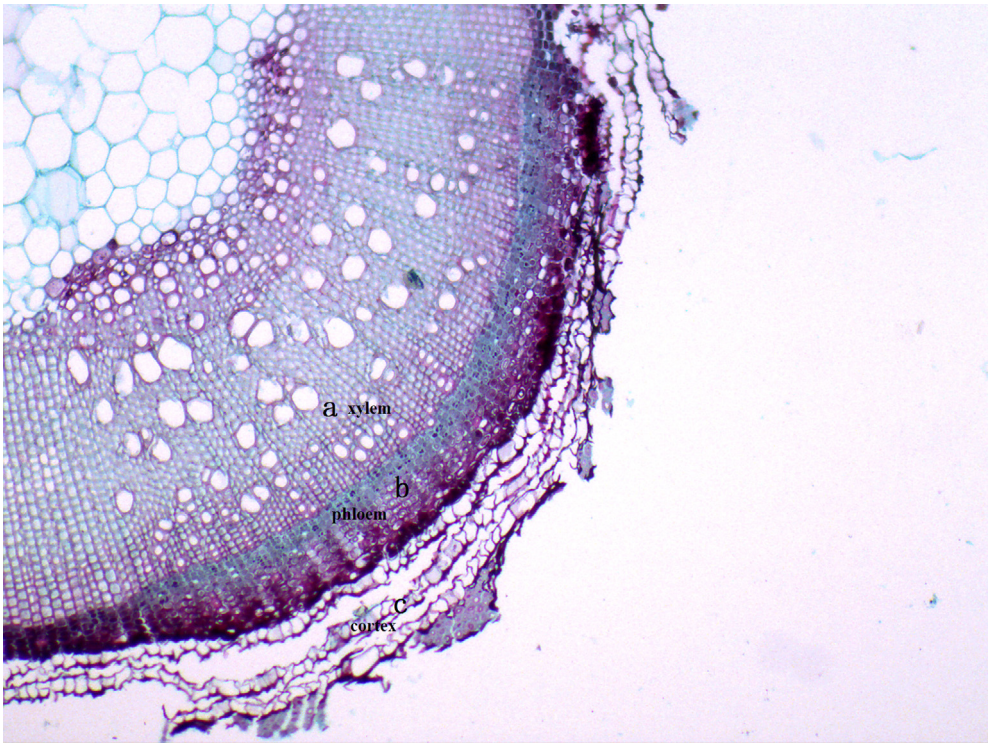

Fig. 2. The paraffin section of living intact *I. rubescens* stem.

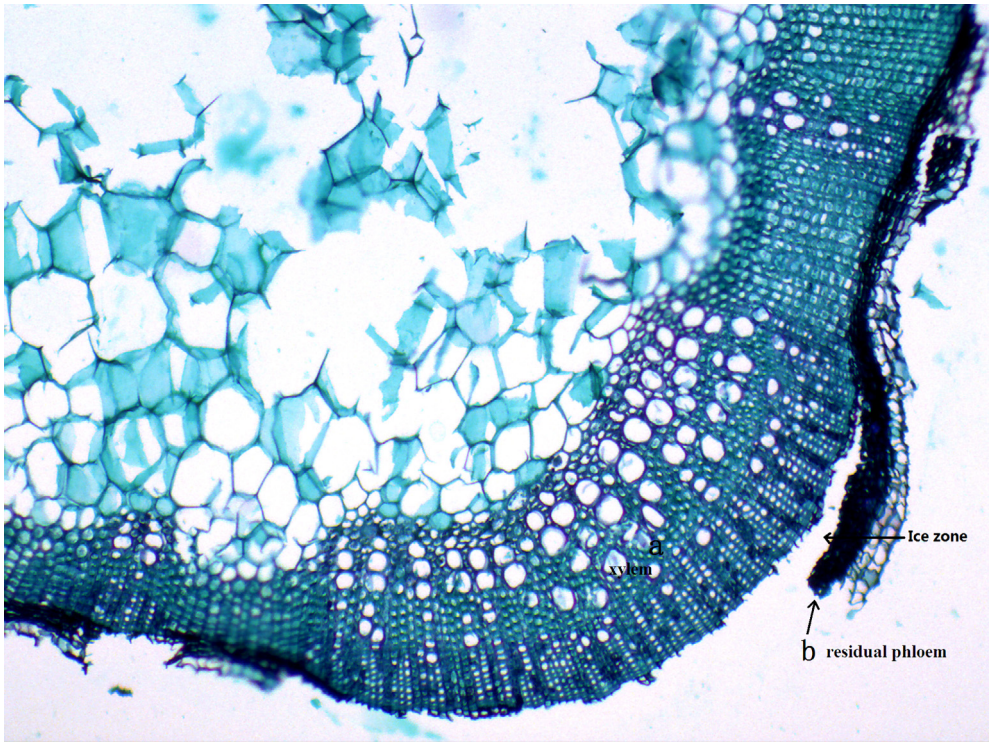

Fig. 3. The paraffin section of *I. rubescens* stem peeled due to ice ribbons.

peeled *I. rubescens* stem and the upper section of stem died soon. There no ice ribbons on *I. rubescen* stems and the barks of stems were intact at air temperature above °C. The *I. rubescens* stems with intact barks did not die.

The paraffin section of intact *I. rubescens* stem was shown as Fig. 2. There was phloem around the xylem in the intact *I. rubescens* stem. The phloem was dyed green and the xylem was dyed red in the paraffin section of intact *I. rubescens* stem. There was imperfect epidermis on the surface of *I. rubescens* stem.

The paraffin section of peeled *I. rubescens* stem was shown as Fig. 3. The ice ribbons were formed between xylem and cambium. The inside cells of cambium were torn by ice ribbons. There was no phloem around the xylem on the ridge of peeled *I. rubescens* stem. The xylem was exposed on the surface of peeled *I. rubescens* stem. There was residual phloem on the ravine of peeled *I. rubescens* stem. The phloem in the detached bark withered.

Some plants have detached bark, such as *Lonicera japonica* Thunb., *Ulmus lamellosa* Wang et S. L. Chang ex L. K. Fu, *Platanus occidentalis* Linn., *Pinus bungeana* Zucc. ex Endl., and *Lagerstroemia indica* Linn. at normal temperature. The detached bark of these plants is entirely made of phellem. There are very few species of plant that peel their barks as the result of ice ribbons at air temperatures below 0 °C. Studies have reported that *Verbesina virginica* and *Helianthemum canadense* develop ice ribbons at air temperatures below 0 °C (Carter 2009; Carter, 2013; Hofmann et al., 2015). These studies did not report whether the stems of these plants died after the ice ribbons formation. *I. rubescens* is a species that peels and withers when ice ribbons arise on its stems at air temperatures below 0 °C in winter. The upper parts of the *I. rubescens* stems would not wither (only the tender tip of stem withered in autumn) in winter if the air temperature was above 0 °C. *I. rubescens* stems peeled and withered because the stems extruded ice ribbons at air temperatures below 0 °C. The ice ribbons separated the phloem from the xylem. The phloem and even the whole bark of the *I. rubescens* stems were torn by the enlarged ice ribbons. The broken phloem could not transport organic compounds. The water in the xylem in the damaged stem could not be transported to the upper stem (Venturas et al., 2017). Therefore, the upper stems of *I. rubescens* died because it receives no water. *I. rubescens* should be cultivated in regions where the air temperature generally stayed above 0 °C to prevent freezing injury. The *I. rubescens* variety that resists ice ribbons should also be bred to expand its cultivation area to cold regions (Li et al., 2011).

4. Conclusions

The phloem was separated from the xylem by the enlarged ice ribbons. The phloem and the whole bark of the *I. rubescens* stems were torn when ice ribbons appeared. The damaged stem could not transport water and nutrition to the upper section. The upper stem above the ice ribbons withered because it could not receives water and nutrition.

5. Interests declaration

The authors of this article declare that they have no conflicts of interest.

6. Abbreviations

Not applicable.

7. Ethics approval and consent to participate

Not applicable.

8. Consent for publication

Not applicable.

9. Availability of data and materials

Data has been permanently archived: https://doi.org/10.5281/zenodo.3830395.

10. Competing interests

The authors declare that they have no competing interests.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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