A Simple Method to Record the Plant Pith Cavity for Field Surveys

Teruo ARASE[1], Taizo UCHIDA[2], Tetsuo OKANO[1], Mayuko KUMAGAI[1]

[1]Faculty of Agriculture, Shinshu University, [2]Faculty of Engineering, Kyushu Sangyo University

フィールドにおける植物の髄腔の簡易記録法について

荒瀬輝夫 [1]・内田泰三 [2]・岡野哲郎 [1]・熊谷真由子 [1]

[1] 信州大学農学部，[2] 九州産業大学工学部

要旨：フィールドにおける植物の髄腔を簡便に記録する方法として「朱肉スタンプ法」を提案するとともに，トクサ（*Equisetum hyemale*）を例にして精度と改善点を検討した。この方法は，茎断面を朱肉につけて紙に印影を記録するものである。茎の外径の異なるトクサ 2 個体を採取し，それぞれ連続した 8 節間の断面について本法を 20 回ずつ試行した。印影をスキャナで画像データに変換し，外径，髄腔径を計測し，皮層厚と髄腔率を算出した。その結果，計測値の誤差はほとんど 0.1 mm 以下であった。髄腔率は 2 個体とも 0.6～0.75 で，節位との関係も類似していた。また，スタンプ成功率は皮層厚との間に有意な曲線関係が認められ，皮層が厚いまたは薄い場合には成功率が低下した。断面の作成とスタンプの仕方に訓練が必要であるが，朱肉スタンプ法は植物の髄腔計測に充分適用できると考えられた。

キーワード：髄腔，朱肉スタンプ法，形態計測，トクサ

Key words: Pith cavity, Imprinting method with cinnabar seal ink, Morphometry, *Equisetum hyemale*

Introduction

A simple method to record the plant pith cavity for field survey will contribute to the investigation of vegetation and enhance the value of herbarium specimens. The plant pith cavity is the hollow space formed by the destruction of the pith in the center of the stem as the plant grows (Shimizu et al., 2001). The width of the pith cavity can be a criterion for species taxonomy in some taxa, e.g. the genus *Equisetum* (Nakaike, 1975; Brume et al., 2008) and some genera of the Asteraceae (Shimizu 2003). However, it is not sufficient to observe the morphology of the pith cavity in herbarium specimens, because pith cavities are flattened in the process of drying and pressing the specimens between sheets of moisture absorbing paper.

There is little prior information on how to record plant pith cavities. In the morphometry of animal bones, there are some reports on the cavities using simple X-rays, computed tomography, and dual-energy X-ray absorptiometry (Hidaka et al., 1998; Hara et al., 1999; Yokoi and Gesso, 2007). Sonic waves are also applied to diagnose the presence of hollows in trees (Nagaishi et al., 1997), despite their rough precision. However, these methods are not suitable for field plant surveys, especially of small herbs, considering the cost and equipment. A microscope with a digital camera seems useful, but there remains a practical problem; it is not easy to set a stem section with a scale at the same level, and to take a photo accurately from directly above.

In this paper, we present a simple imprinting method using cinnabar seal ink to record plant pith cavities for field survey. We also discuss its accuracy and the necessity of improvement from the experimental data using scouring rush (*Equisetum hyemale* L).

Material and Method

Samples of scouring rush were collected in December 2010 from the waterfront of a biotope constructed on the campus of the Faculty of Agriculture, Shinshu University (Minami-minowa Village, Nagano Prefecture, Japan). Their original habitat was the forest floor of a larch forest by the Kamiya River near the campus.

One individual with fine stems and another with thick ones were selected, and one stem of average size was extracted from each individual. We used the third to the tenth internodes, excluding the shortened ones at the bottom of the stem.

The internode was cut by a sharpened cutter knife at the middle, and the water was wiped off with tissue. Then the section was put on a cinnabar seal ink pad and pressed on a Kent paper. The cinnabar seal ink pad we used is a commonly distributed stationery type (Sachihata Co. Ltd., Compact type No. 30; the ink dries in about 3 seconds according to the official statement). Each section was pressed 20 times (20 times × 8 internodes × 2 individuals = 160 patterns in total), in order to grasp the accuracy and the success rate of this method.

An image of the imprint of the section was captured at a resolution of 400 dpi (i.e. 0.0625 mm per dot) with a scanner. Then, the outer diameter and pith cavity diameter were measured with Motic Images Plus 2.0S image processing software, and the cortex thickness and the pith cavity rate against the stem were calculated. We defined the image as a successful imprint if the outer and pith cavity diameters were both measurable from two rectangular angles. Then we calculated the success rate from 20 measurements for each section.

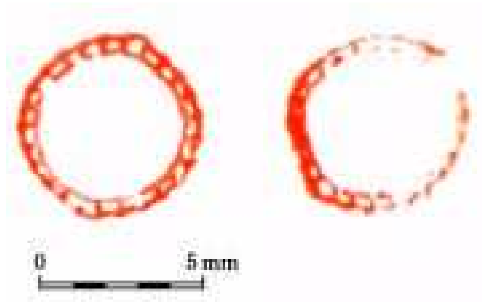

Fig. 1 Images of the stem sections of *Equisetum hyemale* obtained by imprinting method with cinnabar seal ink. Left side: successful imprint, right side: failed imprint.

For data analysis, the success rate of imprint was compared with other variables after an arcsine transformation, because this transformation theoretically approximates a binomial distribution to a normal distribution.

Results and discussion

The images of the sections of scouring rush stem obtained by the imprinting method with cinnabar seal ink are shown in Fig. 1. We could even detect the minute vacant spaces in the cortex in successful images (left side of Fig. 1), whereas some breaks and skews, probably caused by a mistake of pressing direction, could be observed in failed images (the right one). In the preliminary experiments with this method, we observed that the imprints were blurred and ummeasurable when water stayed on the section.

Table 1 shows the measurements for each section of the internodes. The standard deviations (σ), which is a measurement of the accuracy of the images within 20 trials, were mostly under 0.1 mm and had a maximum value of 0.15 mm, i.e. 1–2.5 dots at a resolution of 400 dpi. This result implies that we successfully imprinted the morphology of each section almost in it’s original form and precisely; therefore, our imprinting method can be applied to

Table 1　Measurement of and calculated results from images of stem sections of *Equisetum hyemale*. Each sample size is the number of successful imprints within 20 trials (*n* ranged from 8 to 19).

individual	internode No.	outer diameter (mm) average	outer diameter (mm) σ	pith cavity diameter (mm) average	pith cavity diameter (mm) σ	cortex thickness (mm) average	cortex thickness (mm) σ	pith cavity rate average	pith cavity rate σ
A	3	4.678	0.098	2.823	0.085	0.914	0.048	0.604	0.019
	4	4.721	0.065	3.072	0.066	0.821	0.032	0.651	0.012
	5	4.796	0.075	3.387	0.044	0.702	0.035	0.706	0.010
	6	4.610	0.061	3.284	0.040	0.662	0.031	0.713	0.011
	7	4.402	0.077	3.267	0.056	0.567	0.032	0.742	0.012
	8	4.085	0.078	2.992	0.067	0.546	0.039	0.733	0.016
	9	3.568	0.093	2.584	0.077	0.488	0.035	0.724	0.017
	10	3.083	0.066	2.274	0.102	0.406	0.031	0.737	0.022
B	3	6.302	0.099	3.951	0.101	1.176	0.091	0.627	0.024
	4	6.477	0.074	4.221	0.153	1.128	0.092	0.652	0.026
	5	6.443	0.110	4.250	0.108	1.096	0.068	0.660	0.018
	6	6.389	0.083	4.490	0.074	0.949	0.024	0.703	0.007
	7	6.285	0.084	4.621	0.060	0.832	0.053	0.735	0.014
	8	5.957	0.085	4.348	0.070	0.805	0.042	0.730	0.012
	9	5.656	0.097	4.294	0.057	0.681	0.036	0.759	0.010
	10	5.412	0.103	4.160	0.074	0.626	0.048	0.769	0.015

measure the outer and pith cavity diameters of plants, at least scouring rush (in the range from 2 to 7 mm). However, cortex thickness is so thin (from 0.4 to 1.2 mm) that a dot in the image is of greater consequence than to outer and pith cavity diameters. For more precise measurement, higher-resolution image capture will be needed.

The relationship between outer diameter and pith cavity rate is shown in Fig. 2. Pith cavity rates ranged from 0.6 to 0.75 independently of the difference in outer diameter. The change of pith cavity rates along the internode (No. 3, 4, ···, 10) also showed common trends in each thick or thin stemmed individual; pith cavity rate increased up to the 7th internode and reached a peak after the 8th. Since the pith cavity rate is expressed as fraction of the outer diameter in the taxonomy of genus *Equisetum* (Nakaike, 1975), recording the pith cavity by our imprinting method with cinnabar seal ink is considered to be sufficient to identify species.

The success rate of imprinting was not significantly related to the outer diameter, pith cavity

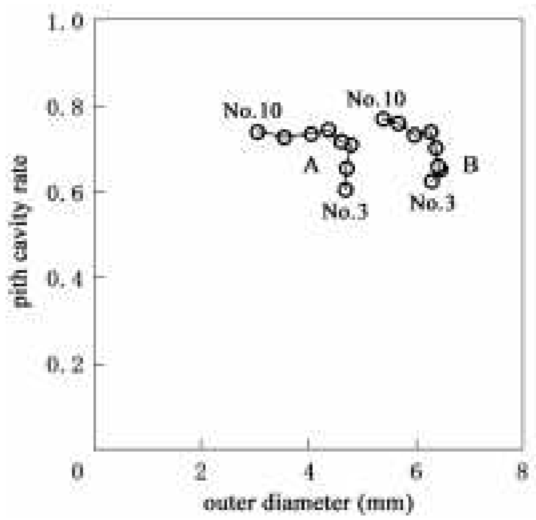

Fig. 2　Relationship between outer diameter and pith cavity rate in *Equisetum hyemale* stems. A and B correspond to Table 1. No. means the number of internode from the base.

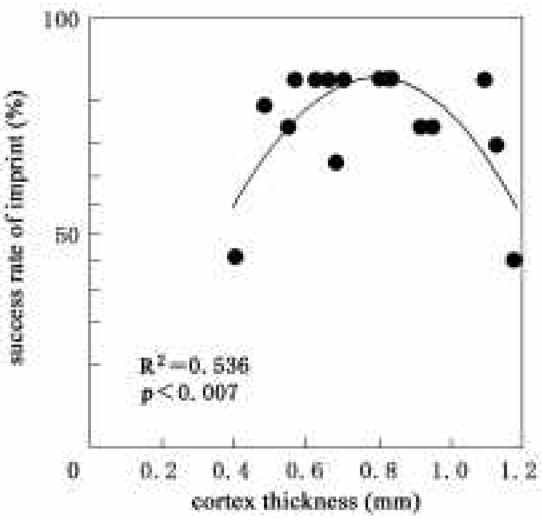

Fig. 3　Relation between the success rate of imprint and cortex thickness. Vertical axis is arranged for an arcsine transformed scale.

diameter or pith cavity rate (r =0.106, 0.209 and 0.203, respectively). However, it can be seen from Fig. 3 that the rate had a marked curvilinear relationship to cortex thickness, and we obtained a statistically significant quadratic regression expression (R^2 =0.536, p <0.007) with a peak at 0.8 mm of cortex thickness. This indicates that the breaks and skew points are apt to be caused when the material is excessively thick or thin. In the case of thick material, the problem might be in cutting process; it is difficult to cut the sections exactly in the same plane. In the case of thin material, those breaks and skew points are conceivably caused in imprinting; the section is susceptible to bent because of its low geometrical moment of inertia. (Higuchi and Saito, 1966). Therefore, training in this method is necessary to make sections evenly and press them without any transformation of the section. For the same purpose, other items such as the instrument used to cut, the sort of paper used for imprinting and the base to set the papers on are worth considering because they may influence the success rate.

As mentioned above, the imprinting method with cinnabar seal ink reproduces stem sections of scouring rush accurately. Cinnabar seal ink pads are quite commonly distributed because they are used for important documents in everyday life in place of signatures in Japan (Ichiki et al., 1996). Murao (2001) presented a method with cinnabar seal ink in science education to estimate the impulsive force by measuring the contact area when a ball with the ink collides with floor. Since the method in this paper is reasonable, simple and accurate, it can be applied not only to morphometry of plants, but also to teaching material.

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