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Cissus quadrangularis L. 及其它小型肉质植物标本制作中的压制前处理*

GITURU Wahiti Robert　王青锋**　王　勇　郭友好

(武汉大学生命科学学院, 武汉　430072)

摘　要: 以*Cissus quadrangularis* L. 为材料用不同的技术处理并比较其收缩程度、色彩保真程度以及茎附属物的保留情况。试验了 3 种常规预处理方法及 2 种针对肉质组织的处理方法, 并进行 ANOVA(Analysis of variance) 分析, 结果显示 3 种常规植物标本预处理方法明显不同($P < 0.005$), 用 LSD(least significant difference) 分析方法分析得出深冷冻法对植物标本造成的损害比汽油法(LSD = 1.708) 和福尔马林法(LSD = 2.065) 要小; 汽油法和福尔马林法处理后得到的标本质量无明显差别。用 ANOVA 法分析醋酸法、纵切法得到的标本及未经任何处理的标本, 它们的效果也有明显不同($P < 0.001$), 醋酸法得到的标本比纵切法 (LSD = 2.371) 和未经任何处理得到的标本 (LSD = 2.138) 效果更好, 而纵切法和未经任何处理得到的标本并无明显不同(LSD = 0.233NS)。醋酸处理法制作小型肉质植物标本其效果得到了植物标本馆工作者的肯定。

关键词: *Cissus quadrangularis*; 标本馆技术; 肉质植物

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Pre-Pressing Treatment for *Cissus quadrangularis* L. and Other Small Succulents

GITURU Wahiti Robert, WANG Qing-Feng**, WANG Yong, GUO You-Hao

(*College of Life Sciences, Wuhan University*, Wuhan　430072, China)

Abstract: Herbarium specimens of *Cissus quadrangularis* L. were prepared by different techniques and compared for shrinkage, retention of color, and persistence of stem appendages. The efficacy of three pretreatment techniques and two methods of handling succulent tissue were investigated. Using analysis of variance (ANOVA) there was shown to be a significant difference ($P < 0.005$) between the three pretreatment techniques. Using the least significant difference(LSD) test, the deep-freezing technique was shown to produce less distortion in the resultant specimens than the gasoline technique (LSD = 1.708)

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作者简介: GITURU Wahiti Robert(1967-), 男, 肯尼亚人, 博士, 从事系统与进化植物学研究。

** 通讯作者。

and the formalin technique (LSD= 2.065). No significant difference was observed in the quality of specimens produced by the formalin and the gasoline techniques (LSD= 0.357NS). ANOVA test indicated a significant difference in efficacy between the acetic acid treatment, the longitudinal sectioning treatment and untreated specimens ($P< 0.001$). The acetic acid pretreatment was shown to be superior to the longitudinal sectioning pretreatment (LSD = 2.371) and untreated specimens (LSD= 2.138). No significant difference was found between the untreated specimens and those subjected to longitudinal sectioning (LSD = 0.233NS). The acetic acid pretreatment technique is recommended for treating specimens of small succulents by herbarium workers.
Key words: *Cissus quadrangularis*; Herbarium technique; Succulent plants

Good herbarium specimens of succulent plants cannot be prepared by applying ordinary drying methods without some special treatment[1]. Special techniques are required in order to preserve the shapes of specimens as far as possible and to make them dry reasonably rapidly. The authors have experienced considerable difficulties in the preparation of herbarium specimens of succulent plants. They recognized a need for better methods both for the speedy killing of the plant material to stop growth while in the press and for pre-pressing techniques that deal with the excessive succulent tissue. If these procedures are not followed in drying succulents, the stems stay alive in the press for many months, and in the process, abscission layers form and cause the leaves, flowers and fruits as well as other stem appendages to fall off. Eventually fungi attack the specimens and they blacken with mold and finally rot.

Improved methods designed to produce better specimens have been suggested[2-5]. Different methods have been used for killing specimens of succulent plants prior to pressing with different degrees of success. Investigations are required to identify the most appropriate method of killing succulent plant materials. The desirable method should result in herbarium specimens with little distortion and adequate retention of the natural colors of the plant parts. Excessive detachment of stem appendages such as tendrils and flowers must also be avoided.

Logan[6] developed a pre-pressing treatment technique that was found to be suitable in hastening drying of specimens of *Begonia* species and some succulents. The technique involves soaking the specimens in a dilute solution of acetic acid laced with a few drops of detergent. This technique has not yet been tested on *C. quadrangularis*. It is also necessary to compare the herbarium specimens obtained by this technique with those obtained from material prepared by the conventional methods used for specimens of *C. quadrangularis*.

1 Materials and methods

1.1 Fieldwork

Localities in Kenya for specimens of *C. quadrangularis* recorded in the East African

herbarium, Nairobi (EA) were visited. The populations of the species were observed in the wild, and living material was collected. Relevant information that goes with a herbarium specimen including the altitude, growth habit, color and scent of flowers, color of leaves and stems, and the color of young and ripe fruits was recorded.

1.2 Voucher specimens

Materials for voucher specimens was preserved in two ways, namely as spirit collections and as pressed specimens. Spirit collections were prepared by storing specimens in a mixture of formaldehyde-alcohol-acetic acid (FAA) in the ratio of 3∶6∶3. Pressed specimens were prepared following the methods of Forman and Bridson[7]. Voucher specimens in this study were deposited in the Kenyatta University herbarium and the Wuhan University herbarium (WH).

1.3 Specimen pretreatment techniques

1.3.1 Killing plant specimens

To compare the different pretreatment methods used for killing plant specimens before pressing, the following procedure was adopted.

Dimensions of various plant organs were measured and recorded from thirty specimens of *C. quadrangularis*. Measurements were made with a sensitive vernier calliper with a sensitivity of 0.002 mm. This was done immediately after gathering to ensure that the material was still fresh and minimize errors arising from shrinkage. The dimensions measured included: stem (width of one face), node (width of one face), internode length, tendril diameter, length of leaves and width of leaves.

(a) Ten specimens were placed in the freezing compartment of a refrigerator for 2 hours and then removed and allowed to thaw.

(b) Ten specimens were dipped in petrol for about 12 hours and then removed and the petrol was allowed to evaporate.

(c) Ten specimens were immersed in 40% formalin for about 2 hours and then removed and the formalin was allowed to evaporate.

(d) The specimens were placed individually in plant presses. The blotters and absorbent papers were changed every day until no more damp spots were observed on the blotters. For the purposes of the experiment, the specimens were kept in the presses for one month. The specimens were observed and re-measured after the one-month period to determine dimensional changes. They were also observed for retention of color and presence of detached pieces e.g. leaves and tendrils. The experiment involved six treatments with each treatment having 10 replicates.

1.3.2 Dealing with succulent tissue

Measurements of organ dimensions were obtained from 30 specimens as in step 1.3.1 above. The specimens were placed in the freezing compartment of a refrigerator for 2 hours and then removed and allowed to thaw.

(a) Ten of the specimens were soaked for 48 hours in 2 liters of 5% acetic acid into which four drops of detergent had been added.

(b) Ten of the specimens had their stems cut longitudinally to cut through the waterproof cuticle.

(c) All the thirty specimens were placed individually in plant presses. The blotters and absorbent papers were changed every day and, for each specimen, the number of days taken before damp spots disappeared from the blotters was recorded. For the purposes of the experiment, the specimens were kept in the presses for one month. The specimens were observed and re¬measured after the one ¬month period to determine the dimensional changes. They were also observed for retention of color and presence of detached pieces e. g. leaves and tendrils. The experiment involved six treatments with each treatment having 10 replicates.

2 Results

2.1 Pretreatment techniques

The results of dimensional changes occurring in the herbarium specimens prepared from specimens of *C. quadrangularis* killed by three different methods are summarized in tables 1 and 2.

表1 3种不同的固定方法下10份 ***C. quadrangularis*** 标本的器官尺寸平均变化百分数和干燥所需的平均天数(这些标本在处理之后压制了1个月)

Table 1 Mean percentage organ dimension changes and mean time required for drying (days) of 10 specimens of *C. quadrangularis*

Organ dimension	Pretreatment		
	Deep freeze	Gasoline	Formalin
Stem diameter	2.89	20.68	17.31
Node diameter	3.56	11.53	13.63
Internode length	0.60	3.05	3.40
Tendril diameter	3.08	31.82	32.29
Leaf length	21.65	29.81	21.61
Leaf width	19.36	23.53	37.94
Drying time(d)	9.8	10.1	10.6

表2 3种不同预处理方法下的 **ANOVA** 表

Table 2 Analysis of variance(ANOVA) table for comparison between three pretreatment techniques

Sources of variation	Sums of squares	Degrees of freedom	Mean sums of squares	*F*
Treatment	216.435	2	108.218	83.502
Organs	14.623	5	2.925	2.257
Error	14.257	11	1.296	
Total		18		

Treatments: Tabular value at degrees of freedom 11, 2= 13.81; $P< 0.001^{***}$.

Organs: Tabular value at degrees of freedom 11, 5= 3.20; $P> 0.05$.

LSD: A. Freezing method= 2.142, A- B= 1.708^{**};
B. Gasoline method= 3.85, B- C= 0.357 NS;
C. Formalin method= 4.207, A- C= 2.065^{**}.

2.2 Specimen shrinkage and appendage retention

The results of dimensional changes occurring in herbarium specimens prepared from specimens of *C. quadrangularis* subjected to two different specimen pretreatment techniques as well as from untreated specimens are summarized in tables 3 and 4. Figure 1 (A) shows a representative specimen treated by deep freezing at - 4℃ for 2 hours before pressing. Figure 1(B) shows a representative specimen subjected to the same pre¬pressing treatment as specimen (Fig. 1: A) followed by soaking in 5% acetic acid for 48 hours before pressing. The former specimen is characterized by extensive detachment of

appendages and discoloration of the inflorescences. The latter specimen displays markedly superior retention of appendages.

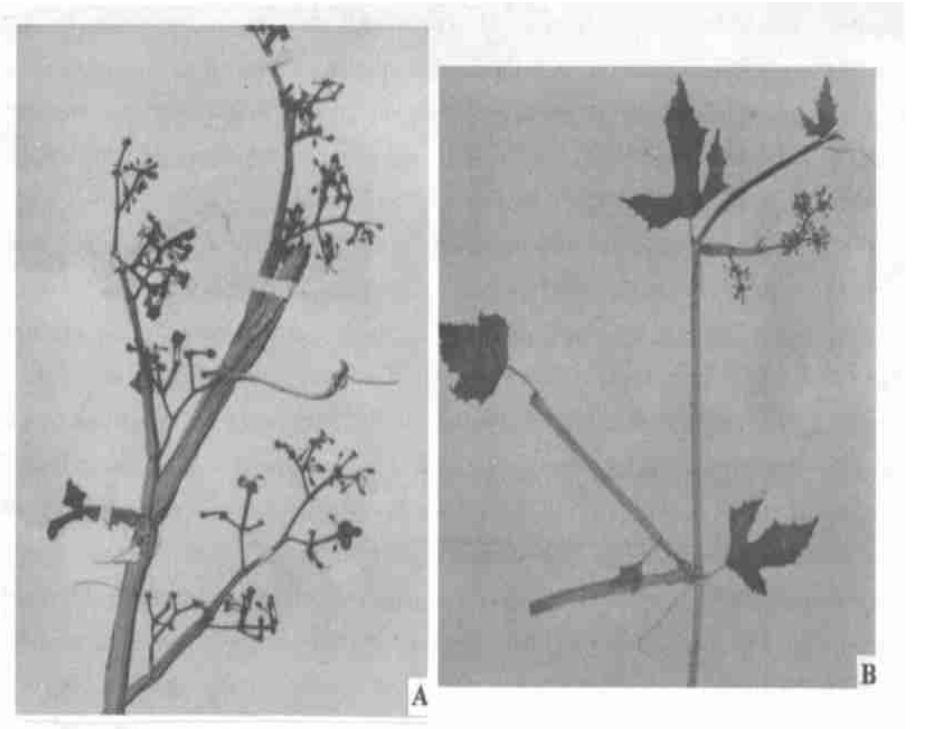

图 1　**A**. 用 - 4℃低温处理 2 **h** 之后压制得到的一个具代表性的标本(Gituru 22) (×0.4) (请注意叶片及茎卷须的脱落和花序的褪色情况); **B**. 用 - 4℃低温处理 2 **h** 之后再用 5% 醋酸处理 48 **h** 得到的结果(Gituru 24) (×0.6) (请注意叶片及茎卷须并未脱落)

Fig. 1　A. A representative specimen of *C. quadrangularis* (Gituru 22) treated by deep freezing at - 4℃ for 2 hours before pressing (×0.4) (NB the detachment of leaves and tendrils and discoloration of the inflorescences); B. Representative specimen of *C. quadrangularis* (Gituru 24) treated by deep freezing at - 4℃ for 2 hours followed by soaking in 5% acetic acid for 48 hours before pressing (×0.6) (NB the presence of leaves and tendrils which have not become detached)

表 3　3 种不同的预处理方法下 10 份 ***C. quadrangularis*** 的器官尺寸平均变化百分数和干燥所需的平均天数(这些标本在处理之后压制了 1 个月)

Table 3　Mean percentage organ dimension changes and mean time required for drying (days) of 10 specimens of *C. quadrangularis*

Organ dimension	Technique		
	Longitudinal sectioning	Acetic acid treatment	Untreated specimens
Stem diameter	32.04	11.28	16.04
Node diameter	13.53	7.94	9.07
Internode length	2.97	0.03	4.05
Tendril diameter	28.33	26.59	33.56
Leaf length	8.37	1.39	8.82
Leaf width	21.54	12.54	22.91
Drying time	7.5	6.8	9.8

表 4　3 种用来处理肉质植物组织的方法比较后得出的 **ANOVA** 表

Table 4　ANOVA table for comparison of 3 different methods of dealing with succulent tissues

Sources of variation	Sums of squares	Degrees of freedom	Mean sums of squares	*F*
Treatment	20.486	2	10.243	7.786
Organs	117.237	5	23.447	17.824
Error	13.155	10	1.315 5	
Total		17		

Treatments: Tabular value at degrees of freedom 10, 2 = 7.786; $P < 0.05^{*}$.

Organs: Tabular value at degrees of freedom 10, 5 = 17.824; $P < 0.001^{***}$.

LSD: A. Longitudinal sectioning A - B = 2.371**; B. Acetic acid pre-pressing treatment A - C = 0.233 NS; C. Untreated specimens B - C = 2.138**.

3 Discussion

In preparation of herbarium specimens of *C. quadrangularis* the method of killing plant material by freezing has been found to produce herbarium specimens that are superior to those obtained from material killed by immersing in formalin or gasoline. Freezing resulted in herbarium specimens with less distortion. In addition, the specimens obtained showed greater retention of natural colors and less tendency of detachment of pieces such as tendrils, leaves and flowers.

It is common practice for some botanists to use formalin for killing specimens of succulent material in the field before placing them in plant presses. This study has shown that the use of gasoline eventually produces specimens that have less distortion than those obtained from specimens treated with formalin. This finding is especially important since it could have serious implications for field botanists who are frequently encumbered by having to carry containers of FAA to the field. Gasoline would be more readily available under these conditions since it is carried as part of the emergency supplies of motor vehicles. In addition, gasoline is more commonly available than "odorless carrier" and unlike o/c gasoline evaporates rapidly from the plant material. This eliminates the need to hang up material before pressing which is necessary for material treated with o/c. The volatile highly inflammable nature of gasoline makes it necessary to avoid working in closed places and near any sources of fire. These conditions, while not being particularly difficult to achieve, especially under field conditions, present serious limitations to the widespread use of this technique. In spite of producing good results, the use of microwaves for killing and drying specimens of succulent plants is unlikely to become widespread in developing countries. This is largely due to the high cost of the equipment required for this technique.

The pre-pressing treatment technique developed for *Begonia* species by Logan[6] has been found to be effective for preparing specimens of *C. quadrangularis* and other small succulents. A soaking period of 48 hours in two liters of 5% acetic acid laced with 2 drops of detergent was found to be satisfactory. Compared to untreated specimens as well as those treated by longitudinal sectioning before pressing, the specimens subjected to the acetic acid treatment dried faster, showed less distortion and eventually retained more natural color after pressing. The use of the acetic acid technique, in combination with the technique of killing specimens by freezing, results in specimens of superior quality and could reduce considerably the cost of preserving the specimens in herbaria. This is particularly important in many developing countries where herbaria have been facing considerable budgetary constraints.

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