Proceedings of the Iowa Academy of Science

Volume 52 | Annual Issue Article 14

1945

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COMPRESSION IN MICROTOME SECTIONS OF PLANT TISSUES

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Compression and other distortions in microtome sections of animal tissues have recently been the object of studies by Dempster (1) and Marengo (2). Dempster stated that compression of animal tissues embedded and cut in 52°C. m.p. "Biloid" paraffin varied from 15% in thick sections (20-25μ) to over 50% in identical material sectioned between 5 and 10μ. He varied the clearance angle of the knife from 1-6°, tried several kinds of paraffin with different melting points and used a number of cutting speeds to determine the amount of compression under routine laboratory conditions. Marengo, however, found that compression was slight in 3-5μ sections of bull testis embedded and cut in 56-58°C. m.p. "Biloid" paraffin to which small amounts of bayberry wax and beeswax had been added. By an ingenious method of direct measurement he demonstrated that compensatory thickening, an index of compression, was practically insignificant when bull testis was cut in hard paraffin with a knife angle of 7°.

These studies upon animal tissues should not be expected to serve as a criterion of similar compression on microtome sections of plant materials. Plant tissues having firm cell walls and including vascular elements not found in animals, might be expected to exhibit less compression. The present study is the beginning of a series of experiments in which attempts will be made to measure compression in microtome sections of various plant tissues.

MATERIALS AND METHODS

In this preliminary study all observations were restricted to transverse sections cut from the matured leaves of *Syringa villosa* Vahl. These leaves averaged approximately 320μ in width in the vein islet areas between major veins. (In this paper "width" refers to the distance between upper and lower surfaces of the leaf; "thickness" designates the microtome setting). Leaves were collected in September, 1944, on the University of Iowa Campus. Rectangular areas were cut from the central portion of each leaf and immediately killed in formalin-acetic-alcohol. Every sample included the midrib and a strip of the blade on each side of it 5-10 mm. wide.

Measurements of these killed tissues were made on transverse free-hand sections carefully cut from the end of the sample nearest the leaf apex. Readings were taken with a calibrated microscope, using a 0.17 mm. objective and a 7x ocular, at two to four locations a specified number of micrometer units from the midrib or from an easily identified major vein. Measurements of such sections were found to approximate very closely those of living material.

When these measurements were recorded, the remaining tissues were dehydrated in butyl alcohol and embedded in 52-54°C. m.p. "Fisher Tissuemat". Each piece was embedded and mounted in such a way that the first microtome section would immediately follow the previously-cut free-hand section. Where the edge of the embedded leaf tissue did not exactly parallel the blade it was necessary to cut only a few sections before one was obtained which included the full width of a desired station.

Mounted paraffin blocks containing the tissues were stored in a refrigerator (at 3°c.) until they were removed for sectioning. This contrasts with Dempsters' experimental procedure of allowing blocks to reach room temperature (20-26°c.) throughout before cutting.

Tissues were sectioned with a Spencer rotary microtome at given thicknesses with the clearance angle of the knife not exceeding 6° and at a room temperature of 24°C. Since it is obviously a time saving, economical practice to do much routine sectioning with safety razor blades, a heavy single-edged safety razor blade in a Spencer holder was used instead of a regular microtome knife. The blade and holder were stored in a refrigerator and maintained at approximately the same temperature as the paraffin blocks. The holder and paraffin blocks were immersed in ice water at intervals as necessary during cutting.

Widths were determined for three series of microtome sections. The first series, as a trial run, was arbitrarily cut at 12μ and compared in width to corresponding free-hand sections cut from the same sample of tissue (Table 1). A second series was cut at 5, 10 and 25μ. Sections of each of these thicknesses were mounted on the same slide and measured at the predetermined stations established in free-hand sections. These were compared in width with corresponding free-hand sections cut from the same sample of tissue (Table 2).

Because the width of thick sections was found to compare closely to that of adjacent free-hand sections, tissues of a third series were cut without preliminary measurements of free-hand sections. Serial sections were cut from each block at 5, 10, 15 and 20μ. Ten 5μ sections and five each of sections 10, 15 and 20μ thick were mounted on a single slide. Changes in width of 5 and 10μ sections of this series were determined by comparison with the corresponding stations in adjacent thick (20μ) microtome sections, rather than to free-hand sections (Table 3). A free-hand paraffin section was subsequently cut with a straight-edge razor immediately following the last 20μ microtome section taken from each block. This section was flattened on the warming table and served as an additional standard of comparison for compression in the microtome sections.

Further, in the third series the average of 200 measurements in material cut at 15μ was identical(319μ) to that of 200 corresponding stations in 20μ sections. The average width of forty 15μ and 20μ sections was only .8% less than in adjacent 25μ sections. Ninety-five 20μ stations averaged 335μ in width, only .01% less than those

in adjacent free-hand paraffin sections (338μ). Ninety-five 15μ stations averaged 331μ in width, or .01% less than the succeeding 20μ stations and .02% less than in free-hand paraffin sections.

A total of 1000 separate measurements was made in the third series alone, four stations being measured on each section, two on each of the lateral halves, one about 400μ and the other approximately 800μ from the last cell at one edge of the midrib. In all, more than 1400 separate measurements were made during the course of this study. An average of these multiple measurements tends to reduce personal error and inaccuracies caused by distortion.

Paraffin ribbons were floated on the slide with a moderate excess of fluid and allowed to flatten undisturbed. Slides were left on the warming plate (at 46°C.) for 24 hours. Tissues were stained with fast green and safranin or with safranin and Delafield's hematoxylin.

RESULTS

Table 1. Changes in Width of Stations in 12μ Microtome Sections of Lilac Leaf in 52-54°C. m.p. paraffin as Compared to Corresponding Stations in Adjacent Free-Hand Sections.

Number of Stations	Average Width of Free-Hand Section	Average Width of Microtome Section	Average Change in Width	% and Type of Average Change
61	304μ	285μ	6% compression	Average 3% decrease in width
18	309μ	323μ	4% expansion	
3	300μ	300μ	no change	

Table 2. Changes in Width of Stations in 5, 10 and 25μ Microtome Sections of Lilac Leaf in 52-54°c. m.p. paraffin as Compared to Corresponding Stations in Adjacent Free-hand Sections.

Sectioned at	Number of Stations	Average Width of Free-hand Section	Average Width of Microtome Section	Average Change in Width	% and Type of Change
5μ	35	348μ	280μ	20% compression	Average 20% decrease in width
10μ	33	335μ	315μ	6% compression	Average 5% decrease in width
	3	269μ	277μ	3% expansion	
	3	350μ	350μ	no change	
25μ	23	350μ	338μ	4% compression	Average 1.5% decrease in width
	8	331μ	342μ	3% expansion	
	6	315μ	315μ	no change	

Table 3. Changes in Width of Stations in 5 and 10μ Microtome Sections of Lilac Leaf in 52-54°c. m.p. paraffin as Compared to Corresponding Stations in 20μ Paraffin Sections.

Sectioned at	Number of Stations	Average Width of 200 20μ Sections	Average Width of Thin Sections	Average Change in Width	% and Type of Change
5μ	400	319μ	254μ	20% compression	Average 20% decrease as compared to 20μ Sections
10μ	100	338μ	303μ	11% compression	Average 2.5% decrease as compared to 20μ sections
	70	319μ	331μ	4% expansion	
	10	281μ	281μ	no change	

DISCUSSION

The work of Dempster and Marengo on compression and other distortions in microtome sections of animal tissues suggests the need for similar studies upon plant materials.

The results given in the present paper are restricted to observations obtained under what may be considered as ordinary laboratory conditions. A microtome knife is the ideal edge for sectioning in paraffin. However, it is probable that many persons have been deterred from volume sectioning of desirable materials by the fact that so much time and care is entailed in maintaining a knife in proper condition. Razor blades in suitable holders are condemned as worthless by a number of workers yet certain advantages may accrue from their use. Good quality, heavy, single-edged blades are always sharp, are easily obtainable, inexpensive, and may be used to cut ordinary or refractory materials without the possibility of damaging a laboriously prepared microtome knife. When a large number of different tissues are to be sectioned daily the average technician may profit by using razor blades, especially if the sections are not to be cut extremely thin or the work is not of a highly critical nature. A comparison of microtome sections of identical plant materials when cut by both a microtome knife and a razor blade is in progress.

The work of Dempster and Marengo deviated from what may be considered ordinary laboratory procedure. Dempster and Marengo used specially prepared microtome knives with bevel angles of 27° and 23° respectively. Dempster cut blocks at room temperature while it is usually recommended that both block and blade be cooled before sectioning. Marengo used a hard paraffin (necessitated by very thin sections) to which had been added small quantities of bayberry wax

and beeswax. In the present study a factory compounded embedding medium, Fisher Tissuemat, was used entirely. This is a standardized product that eliminates many of the uncertainties of sectioning due to variations in paraffin quality.

It was found that expansion which accompanies flattening of leaf tissues may not only partially or wholly counterbalance the compression resulting from sectioning but may be so great as to result in abnormal width of the section. Compared with adjacent free-hand sections of killed tissue, expansion in certain microtome sections was observed as follows: In the first series 23% of the total stations in 12μ sections, 4% excess expansion (Table 1); in the second series 8% of the 10μ sections and 22% of the 25μ sections averaged 3% excess expansion (Table 2); in the third series 39% of the 10μ sections expanded 4% (Table 3). In no case did this compensatory expansion entirely mask compression in 5μ sections cut in 52-54° paraffin, since no station measured as much as the corresponding one in a free-hand or in a thick paraffin section. Dempster estimated that expansion during flattening ranged from 6% in 5μ sections to 2.3% in 25μ sections (embeded in 56-58° paraffin) with a warming plate temperature 10°C. below the melting point of the paraffin used.

Dempster stated that sections cut with a microtome setting of 2.5-5μ were sometimes several times as thick (15.5-11.9μ respectively), because of compression, as those cut with a higher setting. Marengo, using a 56-58° paraffin, found that microtome settings of 3μ and 5μ resulted in sections respectively only 3.31μ and 5.25μ thick. No attempt can be made in this preliminary work to discuss the character of compensatory thickening that may occur in thin sections of plant tissue cut in 52-54° paraffin (Fisher Tissuemat).

From the above results it would appear that in critical studies of transverse microtome sections of mesophytic leaves the measurements usually taken should be corrected as follows: for sections cut at 5μ, adjust as for 20% compression; 10μ, 4-5% compression; 12μ, 3% compression; 25μ, 1.5% compression. It is probable that less compression would occur in 5μ sections if tissues were: (1) embedded in a harder paraffin, (2) cut with a properly prepared microtome knife, or (3) if more refined methods were developed for using razor blades.

SUMMARY

1. Microtome sections of lilac (*Syringa villosa* Vahl.) leaf embedded in 52-54°C. m.p. paraffin (Fisher Tissuemat) were compared in width with adjacent free-hand sections of killed tissue or to the adjusted measurement of thick microtome sections. The decrease in width (compression of section) averaged as follows: 435 stations cut at 5μ, 20%; 220 stations at 10μ, 4-5%; 82 stations at 12μ, 3%; 34μ stations at 25, 1.5% decrease.

2. Two hundred stations in 15μ sections averaged the same width (319μ) as 200 corresponding stations in adjacent 20μ stations. Forty

stations in 15 and 20μ sections averaged only .8% (24μ) less in width than the corresponding stations in 25μ sections.

3. Change in width is fairly uniform in material sectioned between 10 and 25μ, compression ranging from an average of 5% in 10μ sections to 1.5% for those cut at 25μ, with little change in those between 10-25μ in thickness. It thus appears that critical quantitative data involving multiple measurements can be accurately corrected to compensate for changes in width of microtome sections when cut between 10-25μ in thickness. Forsections cut at 5μ adjust as for 20% compression; 10μ, 4-5% compression; 12μ, 3% compression; 25μ, 1.5% compression.

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