POSTER SUMMARY

PITH/FIBRE MEASUREMENT OF SUGARCANE VARIETIES USING A STEREOMICROSCOPE

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Abstract

Pith/fibre ratio is presently used to characterise the millability of sugarcane. The pith/fibre ratio is measured using an instrument that separates the pith from the fibre of shredded cane samples using different sized mesh screens/sieves. The instrument uses water and air for separation. This method relies on complete separation of pith and fibre which is not always achieved, and it also assumes that the densities of pith and fibre are equal. Looking at the pith and fibre in detail under a microscope will give a better indication of the distribution of fibre and pith in sugarcane. The suitability of using a stereomicroscope to measure the pith/fibre content of different cane varieties is considered, and the method and techniques used in the measurement are given. Comparisons between the new method and standard methods are shown. The advantages, disadvantages and applications of this new measuring technique are discussed.

Keywords: pith/fibre ratio, sugarcane, varieties, stereomicroscope

Introduction

The millability of sugarcane is extremely important in the preparation and extraction processes of a sugar factory (Foster and Shann, 1968). When cane is soft it can be shredded too finely, which can cause problems in extracting the sucrose. In milling tandems this cane cannot be fed smoothly into the mills, and in diffusion it can decrease percolation rates and cause flooding. When cane is too hard, the preparation of the cane becomes difficult and the percentage of unopened sugar bearing cells within the cane increases, reducing extraction. The stalk of the cane plant can be divided into two sections, (i) the fibre, which consists of vascular bundles and gives the stalk its strength, and (ii) the soft pith section containing the juice. The strength of cane is largely based on the amount of vascular bundles present and the tensile strength of those vascular bundles (Sockhill, 1958). Measuring the amount of pith and fibre will give an indication of the strength of the cane. At present the pith/fibre ratio is measured in a pith/fibre separator (Chinsamy *et al.*, 2004). This method is not ideal and provides limited information. The use of a stereomicroscope has been investigated to measure pith and fibre in the cane stalk, using image analysis as an alternative method of measurement.

Experimental

Sample preparation
Samples of sugarcane from the South African Sugarcane Research Institute pre-release variety trials were used in the investigations. The cane stalks were wrapped with Scotch tape to prevent them from breaking, after which slices of the stalk (5-10 mm thick) were obtained using a Stanley knife. Other methods of slicing/cutting, such as using a lathe and a saw, proved unsuccessful as they damaged the open surface of the cane. An extremely smooth, clean cut prior to microscopy is required for successful image analysis.

Vascular bundles were contrasted by placing several drops of 1% (w/v) phloroglucinol in 95% (v/v) ethanol on the top surface of the cane sections and, after approximately 2 min, adding 2-3 drops of concentrated HCl. Phloroglucinol stains lignin red, and this reaction was exploited to reveal the vascular bundles in the pith of the stems tested.

Microscopy and image analysis
A Nikon AZ100 stereomicroscope fitted with a LED ring illuminator on a 0.5x objective lens was the most successful method of visualising the relatively large (2-3 cm diameter) cross-sections of sugarcane. The sample was submerged in water in a petri dish to avoid reflections of light off the sample. Colour (24 bit) images were captured with a 5 megapixel digital camera at a resolution of 1280 x 960 pixels. Image analysis of these images was performed using Image Pro-Plus (Media Cybernetics, US).

When the sum of the area occupied by vascular bundles had been measured automatically, the total area of bundles and pith was measured manually, and the percentage area calculated accordingly.

Results and Discussion

The samples showed varied responses to the phloroglucinol stain, in some cases making it difficult to differentiate pith from fibre. Before the area of the vascular bundles could be measured and quantified automatically by the image analysis package, images had to be manipulated to:

- eliminate differences in brightness between the periphery and inner cortex of the stems,
- delineate accurately the stained lignin-rich xylem elements of the vascular bundles, and
- ‘fill’ the areas within vascular bundles so they could be quantified as if they were solid.

Overall, the method that produced the best results was as follows:

1. Extract the green channel from the red-green-blue (RGB) colour images captured.
2. Use a morphological filter to ‘flatten’ any gradient in brightness.
3. Use a background subtraction and Gaussian filters to reduce background (pith) detail.
4. Enhance image contrast.
5. Use various filters to ‘close’ detail inside the bundles.
6. Using watershedding to delineate and separate discrete bundles from each other where these were in close contact.

Figure 1 shows the different stages of the method described. Image A is the raw image captured before image analysis, image B is after green extraction, background subtraction, flattening and contrast enhancing (steps 1 to 5) and image C (step 6) is the final stage of processing (watershedding) prior to quantification of areas occupied by vascular bundles (black dots) and pith (light regions). Note the dark rim of closely-packed vascular bundles that could not be separated, and the slightly faded appearance of fibres in the centre of the stem.

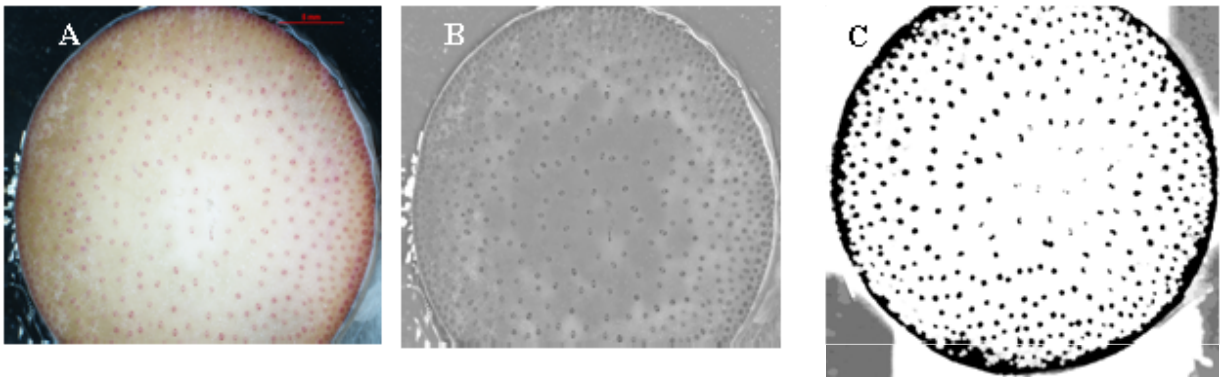

Figure 1. The different stages of image analysis

Although every effort was made to prepare all images to the same optimal level prior to analysis, judgement calls such as the exclusion or inclusion of certain areas means that the present approach is susceptible to operator bias and requires further development. Comparisons made between the two techniques (stereomicroscope vs pith fibre separator) showed a weak linear correlation (% area of vascular bundles occupied = 46 * pith/fibre ratio + 10, $R^2 = 0.41$). The correlation should improve when the method has been refined.

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