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Latex quantification in guayule shrub and homogenate

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Abstract

Commercial development of hypoallergenic latex from *Parthenium argentatum* (Gray), especially for the manufacture of latex medical and household goods, requires specific knowledge on the best lines, agronomic practices and storage conditions for the generation of maximal latex yield. Development can proceed only by using standard, accurate analytical techniques for latex extraction and quantification. In this paper, we describe a latex extraction method that parallels the proposed commercial extraction process and we demonstrate that different extraction equipment can introduce large variations in the apparent amount of extractable latex. An evaluation protocol for testing and optimizing the efficiency of alternative extraction equipment is presented. Also, we describe a rapid latex quantification method for use with shrub and shrub homogenates, confirm its accuracy with tissue balance analysis and show how it can be used to quantify latex in materials with unusually low latex content. The reproducibility of the basic method and of a variation of the method, were determined by analysis of identical homogenates by researchers at different locations. Published by Elsevier Science B.V.

Keywords: Hypoallergenic latex; Latex extraction; Latex yield; *Parthenium argentatum*; Rubber

1. Introduction

Parthenium argentatum (Gray), commonly known as guayule, is a perennial shrub native to the Chihuahuan desert of the US and Mexico. Guayule produces high molecular weight natural rubber, comparable in quality to that currently produced commercially from plantation-grown *Hevea brasiliensis* (Whitworth and Whitehead, 1991; Cornish et al., 1993). The recent widespread occurrence of life-threatening IgE-mediated 'latex allergy' to the protein contaminants in currently available latex products makes the development of a safe, alternative source of natural rubber imperative (Cornish and Siler, 1996a; Nakayama et al., 1996). Latex allergy sufferers and their antibodies do not react to guayule rubber latex (Siler and Cornish, 1994; Carey et al., 1995; Siler et al., 1996), which should, therefore, provide the required source of safe, hypoallergenic, natural

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rubber. Current commercialization efforts are based on the extraction of stable rubber particles from guayule shrub in aqueous suspension and their subsequent purification as a high quality, low protein latex (Cornish, 1996, 1998). Although guayule latex has been characterized (Schloman et al., 1996) and successful prototype latex medical and consumer products have been made (Cornish et al., 1996), true commercialization has not yet been realized. To expedite this goal, additional information on extractable latex content is required to select the best lines for latex yield because selections, prior to hypoallergenic latex production, were based on total rubber content determined by Soxhlet extraction with organic solvents. Total rubber may not represent extractable latex. Also, accurate latex quantification should include latex extraction by a method at least as efficient as the proposed commercial extraction process, be suitable for screening large numbers of plant samples from breeding programs, be able to handle the sample numbers required to optimize agronomic, harvesting and storage practices and give comparable results when used by different researchers.

Kroeger et al. (1996) had developed a latex quantification method based upon the work of Jones (1948). However, this method is time-consuming and requires extensive use of organic solvents. In this paper, we describe a latex extraction process that parallels the proposed commercial extraction process and we demonstrate how it may be applied using different equipment. Also, we describe two variations of a rapid method to quantify the extractable latex in plant tissue as well as the latex concentration in shrub homogenates and demonstrate the reproducibility of the techniques by analysis of identical homogenates by researchers at different locations.

2. Materials and methods

2.1. Plant material

Branches were harvested from mature guayule shrubs that were grown at different field locations in Arizona. Branches were dipped into 1% aqueous ascorbate, sealed in plastic and stored on ice until processed. Some branches were shipped overnight to Albany, CA, while the remainder were kept in Phoenix, AZ.

Harvested branches were sorted into three branch diameters, which approximated the relative age of the different tissues: small, <0.5 cm; medium, 0.5–1.0 cm; large >1.0 cm diameter. The smallest branches contain very little woody material and represent the current year's growth. The medium branches are >1 year old, contain a woody core and have experienced one winter season. The largest branches contain a substantial woody core, are probably at least 2-year-old and have experienced at least two winters. The sorted branches were kept at 4°C until processed.

2.2. Latex extraction

2.2.1. Waring blender method

The sorted, chilled guayule branches were cut into ≈ 1 cm sections. Within 5 min of the first cut, the pieces were immersed in ice-cold, aqueous extraction buffer (0.2% ammonia and 0.1% Na_2SO_3, pH 10) to prevent dehydration of the tissue (1:2, w:v). The branch sections in buffer were ground for 1 min using a Waring blender (model 33BL79, Waring, New Hartford, CT) and filtered through a 1-mm steel mesh filter. Samples of >50 g were pressed through the filter using a 15-cm diameter manual mini-press (Spremi model, D'Errico, Italy), whereas, those below 50 g were filtered through the mesh without the press. Filtration is required before latex quantification to remove woody fragments, which otherwise would float into the latex layer and prevent accurate gravimetric determination of the latex component (see Section 2.3.1). The plant material (bagasse) retained by the filter was then added to the same volume of extraction buffer as before, reground for another 1 min and filtered again. The second grind has the effect of rinsing rubber particles—released from the parenchyma by the first grind, but still trapped in the bagasse—from the material and also of breaking up any remaining intact parenchyma cells. The use of two grinding steps, coupled with filtration, simulates the process (Cornish, 1996) proposed for commercial-scale

guayule processing. The separate filtrates, obtained from the two steps, are normally pooled for subsequent latex quantification. Additional grinding times were initially made to establish the optimal time period. The recommended Waring blender grinding time of 2 min (2×1 min) was based on: (1) visual observation that intact chunks of non-woody tissue had disappeared; (2) particle size analysis of the filtrates using a Horiba LA-900 Laser Scattering Particle Size Distribution Analyzer to characterize and quantify the particulate material < 1 mm diameter (known volumes of homogenate were suspended in known volumes of deionized water to permit quantification); and (3) quantification of the latex in the homogenates. This extraction method, using a single blender, permits one branch sample to be transformed into twice-ground filtered homogenate, ready for latex quantification in ≈ 20 min.

2.2.2. Oster blender method

Within 1 day of harvesting, sorted, chilled samples, consisting of 50 g of branch, were cut into 1 cm lengths and mixed with 50 ml of ice-cold extraction buffer (0.1% Na_2SO_3 adjusted to pH 11 with NH_4OH). Latex was extracted by adding 150 ml of extraction buffer and then, grinding, for 2 min in a 1-l Oster blender (Classic Model, Oster, Hattiesburg, MS). The slurry was filtered through two layers of cheesecloth ($\#50$) in a 100-mm diameter Buchner funnel. The residue in the funnel was pressed using the pressing foot from the mini-press described in the Waring blender method (Section 2.2.1). The pressed residue was then returned to the Oster blender, mixed with 150 ml of chilled extraction buffer (pH 10.5), ground for 1 min and the filtration process repeated. The pooled filtered homogenates were stored at 10°C prior to quantification. Later experimentation showed that these grind times, when used with the Oster blender, were inadequate. Replacing the 2- and 1-min grinds with 4- and 3-min grinds, respectively, adequately extracted the latex fraction from guayule shrub. This extraction method, using a single blender, permits one branch sample to be transformed into thrice-ground filtered homogenate ready for latex quantification in ≈ 45 min.

2.3. Latex quantification

2.3.1. The 1-ml method

The homogenates were first shaken to suspend thoroughly the solid material, including the rubber particles. For each homogenate, 3×1 ml aliquots were pipetted into siliconized microfuge tubes (Daigger, Lincolnshire, IL) and centrifuged in a bucket rotor, with a 60-tube capacity, at $2500 \times g_n$ for 15 min at room temperature, to float the creamy latex fraction to the homogenate surface. (Note: temperatures from 4 to 25°C did not adversely affect the method.) Glacial acetic acid (50 μl) was gently pipetted onto the sample surface, disturbing the latex layer as little as possible. The microfuge tubes were then centrifuged a second time using the same conditions. The coagulated latex rubber was lifted with fine forceps from each tube, rinsed in deionized water, then placed onto preweighed weigh paper. Care was taken to harvest rubber that had adhered to the tube surface. The samples were dried overnight at 37°C, together with three unused weigh papers as blanks. The samples were equilibrated at room temperature for 2 h and then weighed to determine latex yield. This method can be used easily to quantify latex concentrations down to 2 mg dry latex rubber per millilitre of homogenate.

This method permitted latex quantification of 20 homogenized samples, with three replicates, per hour (i.e. a total of 60 determinations per hour, not including sample preparation or drying time).

2.3.2. The 14-ml method

Another version of the method described in Section 2.3.1 was examined, both to demonstrate that the principles of the method are not equipment-specific and also, for use when an available bucket rotor centrifuge is not adapted for use with microfuge tubes. In this variation, a 14-ml aliquot of homogenate was pipetted into a 15-ml tapered plastic centrifuge tube and centrifuged for 15 min at $2200 \times g_n$ in a bucket rotor. The supernatant was transferred into a pre-weighed 15-ml tapered plastic centrifuge tube and centrifuged as before. Glacial acetic acid (0.7 ml) was added to coagulate the latex rubber and the tube was cen-

trifuged a third time under the same conditions. The bottom of the tube was cut using animal-type nail clippers (e.g. model NC464, Four Paws, Hauppauge, NY) and the liquid was allowed to drain from the tube. The coagulated latex component remaining was quantified by vacuum-drying the tube and the cut and cleaned tube bottom, at 60°C to constant weight. This method was used effectively down to 5 mg dry latex rubber per 14 ml of homogenate.

This method permitted latex quantification of 12 homogenized samples, with three replicates, to be analyzed in 5 h (not including sample preparation or drying time).

2.4. Quantification of low latex concentrations

A modification of the 1-ml latex quantification method was developed for samples containing low latex concentrations (< 2 mg/cm^3). A 35-ml sample of homogenate, containing 6.0 mg/ml latex (determined by the 1-ml quantification method) was centrifuged in an SS-34 tube, at $2400 \times g_n$ for 15 min at room temperature. The rubber that floated to the surface was scooped from the tube, dried overnight at 37°C and weighed. This procedure reduced the latex content of the homogenate by 4.67 mg/ml leaving a theoretical concentration of 1.33 mg/cm^3. The low latex homogenate was stirred to resuspend the pellet precipitated during centrifugation and then nutated at 60 rpm for 10 min. Samples of a wide latex concentration range were generated by diluting the original homogenate with (1) buffer and (2) low latex homogenate. Latex content was determined as previously described (Section 2.3.1) and regression analysis was used to determine the concentration in the low latex homogenate (the y-intercept).

2.5. Determination of latex rubber purity

Samples of dried latex, from the 1-ml latex quantification method, were dissolved in 4 ml hexane, in capped scintillation vials, by shaking overnight at room temperature. The samples were vortexed, then filtered through preweighed glass fiber filters (25 mm diameter, Whatman, Fairfield, NJ) to separate out the hexane-insoluble contaminants of the latex pads. The filters were washed by flushing through 3×3 ml aliquots of fresh hexane. The filters were dried at 37°C and weighed to determine the hexane-insoluble component.

2.6. Rubber analysis of shrub and bagasse

The rubber content of samples of the original shrub and of the bagasse resulting from the Waring blender latex extraction method, were determined by the method of Black et al. (1983), which is based on extraction with acetone and cyclohexane.

3. Results

3.1. Optimization of the 1-ml latex quantification method

Because guayule rubber particles are contained within the individual bark parenchyma cells, the particles must be released into an extraction medium from which they can be separated and purified. To accomplish this, the plant material must be ground to rupture all the rubber-containing cells, but without damaging the rubber particles. An unnecessarily prolonged grind could compromise rubber particle integrity, leading to latex loss during subsequent purification procedures through coagulation of damaged rubber particles. Analysis of the particle size distribution of aliquots of homogenate filtered after different grinding times (fraction below 1 mm in size) demonstrated that most tissue fragments were destroyed in 60 s by the Waring blender and no fragments larger than the rubber particle fraction (mean of 1.2 μm) were detectable after 120 s of grinding (Fig. 1). Visual inspection of the tissue retained by the filter showed that fragments larger than 1 mm were composed of woody material.

The latex content of the filtered homogenate sized in Section 3.1, was also analyzed (Fig. 2). The results paralleled the findings of the particle size analysis (Fig. 1) in that most of the latex appeared to have been released after 60 s of grinding and virtually all extractable latex was released by 120 s of grinding.

When the bagasse was reground in fresh extraction buffer, each grind had the additional effect of washing released rubber particles that may become trapped in the bagasse fraction during each filtration step. Therefore, we quantified the latex appearing in the filtrates of a series of 1 min grinds of guayule branches of three different sizes, where fresh buffer was used for each grind (Fig. 3), using the quantification method described in Section 2.3.1. Asymptotic equations were fitted to two sets of data to determine the maximum

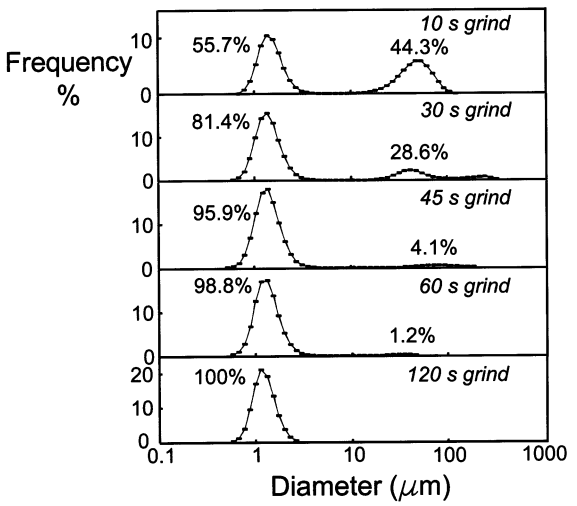

Fig. 1. Particle size distribution of guayule homogenates, filtered through 1-mm mesh, after branches (0.5–1.0 cm in diameter) were ground for different times. Particle size was determined using a Horiba LA-900 Laser Scattering Particle Size Distribution Analyzer. Percentages in the figure panels reflect the relative proportions of particulate material in the above and below 10 μm diameter classes.

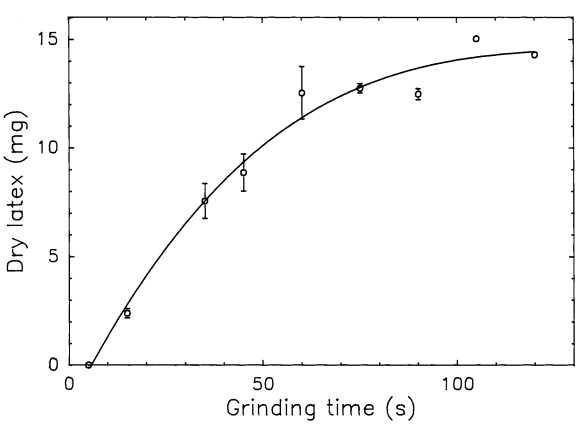

Fig. 2. Rubber extracted as latex from guayule shrub branch homogenates, filtered through 1-mm mesh, after branches (0.5–1.0 cm in diameter) were ground for different times. Each value is the mean of $3 \pm$ S.E.

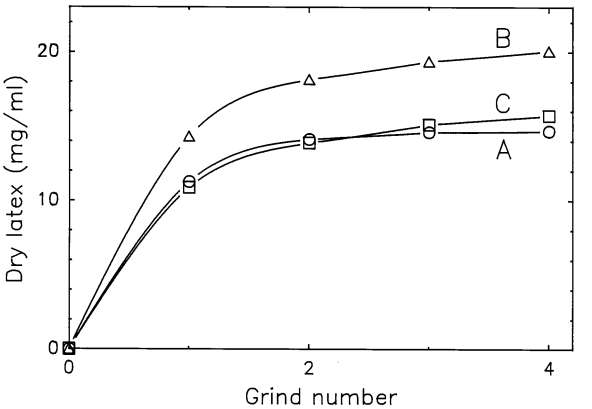

Fig. 3. Accumulation of rubber extracted as latex from homogenates of three different guayule shrub branch sizes, as a function of the number of 1-min grinds. Branch diameters were (A) ○, ≤ 0.5 cm; (B) △, 0.5–1.0 cm; (C) □, >1.0 cm. Each value is the mean of three determinations.

Table 1
Amount of extractable latex obtained from three different sizes of guayule branches, after a series of 1-min grinds using a Waring blender[a]

Batch	Grind No.	Batch diameter (cm)		
		≤0.5	0.5–1.0	>1.0
		Latex extracted (%)		
1	1	78.0	71.0	68.4
	2	95.1	91.6	90.0
	3	98.9	97.6	96.8
	4	99.8	99.3	99.0
2	1	80.4	72.2	74.4
	2	96.2	92.3	93.4
	3	99.3	97.8	98.3
	4	99.9	99.4	99.6

[a] The homogenate was filtered through a 1-mm steel mesh after each grind and the bagasse was then reground with fresh buffer. The percentages are estimated from the asymptotic analysis of the values shown in Fig. 3 and similar extractions of a second batch of guayule branches. (See Table 2 for the asymptotic equations and comparison with the experimental data).

amount of extractable latex in the different branch sizes. Analyses of the data demonstrated that at least 99% of the latex could be effectively extracted by the fourth 1-min grind, with at least 90% being released by the first two grinds (Table 1), irrespective of the initial branch diameter of the tissue extracted. The latex asymptotes closely agreed with the experimental data and so were used to calculate the latex content in the original branches (Table 2).

The filtration step was also examined further, to determine whether the use of pressure to force the filtrate through the 1-mm steel mesh (which requires volumes of homogenate over 50 g) gave different latex values to unpressed filtrates (for homogenate quantities below 50 g). Three branch sizes and the 4 or 5×1 min grinds were analyzed (Fig. 4), but very little difference in the two filtration methods was discerned, indicating that any filtration method that removed the woody bagasse prior to latex quantification would be effective.

To determine whether the latex extraction or quantification methods introduced any apparent rubber losses from the tissues, the latex and the residual rubber in the bagasse were determined over a series of 4×1 min grinds of two different batches of guayule shrub divided into three branch sizes. Total rubber remained constant over the four grinds (Table 3).

The latex was quantified after extraction from three different branch sizes in separated filtrates from the 2×1 min grinds and from samples in which the first and second grind filtrates were pooled before latex quantification. The experimentally determined pooled values were comparable to the pooled values calculated from the separate filtrates (Table 4). Additional experiments demonstrated that the 1-ml quantification method was not perturbed by the presence of the leaves when the shrub samples were initially ground (data not shown).

3.2. Latex quantification of low concentrations

Using our 1-ml latex quantification method, the latex content of low latex homogenate was 1.30 ± 0.10 mg/dw per cm^3, which agrees extremely well with the predicted value of 1.33 determined from subtraction of the weight of the latex removed from the standard homogenate (see Section 2.4). When a range of latex concentrations was prepared in an otherwise identical homogenate background and then quantified, a linear relationship was observed (Fig. 5, (○), $r = 0.9996$). The accuracy of the method is confirmed by the y intercept

Table 2
Latex yield from guayule branches showing actual extracted latex (each value the mean of three determinations with the 1-ml quantification method), maximum latex yield calculated from the asymptotes (which estimate 100% extractable latex) and the original latex content of the branches before extraction, calculated from the asymptotes[a]

Batch	Branch diameter (cm)	Actual latex (mg/ml)	Latex asymptote (mg/ml)	Latex in original branches (% dry weight)
1	≤0.5	21.24	21.13[b]	8.64
	0.5–1.0	25.90	25.90[c]	9.73
	>1.0	21.87	21.70[d]	7.82
2	≤0.5	14.42	14.55[e]	5.02
	0.5–1.0	20.05	19.98[f]	6.66
	>1.0	15.70	15.66[g]	5.17

[a] All values are based upon the amount of latex released from four 1-min grinds.
[b] Asymptotic equation: $21.13(1-e^{-1.63*\text{grind number}})$.
[c] Asymptotic equation: $25.90(1-e^{-1.28*\text{grind number}})$.
[d] Asymptotic equation: $21.70(1-e^{-1.36*\text{grind number}})$.
[e] Asymptotic equation: $14.55(1-e^{-1.51*\text{grind number}})$.
[f] Asymptotic equation: $19.98(1-e^{-1.24*\text{grind number}})$.
[g] Asymptotic equation: $15.66(1-e^{-1.15*\text{grind number}})$.

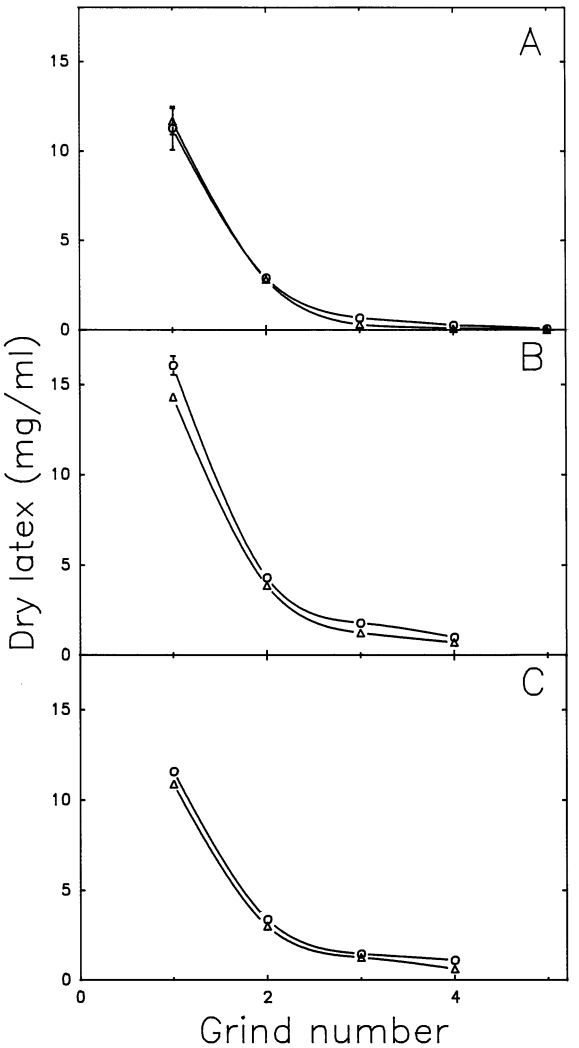

Fig. 4. Comparison of rubber extracted as latex from homogenates filtered with (△) and without (○) pressure through a 1-mm steel mesh screen, after each of four 1-min grinds. Branch diameters were (A) ≤ 0.5 cm; (B) 0.5–1.0 cm; (C) > 1.0 cm. Each value is the mean of $3 \pm$ S.E.

of 1.27 mg/ml from the regression analysis, which reflects the latex concentration in pure low latex content homogenate. This value of 1.27 mg/ml is again very similar to the predicted value of 1.33 and the determined value of 1.30. Samples with < 2 mg/ml of latex are difficult to quantify accurately by themselves (and samples of < 1 mg/ml of latex are impossible to quantify accurately) because lower amounts produced a fragmented coagula difficult to collect with the forceps. Nonetheless, we have shown that concentrations considerably below 2 mg/ml can be quantified by determining the y intercept from a regression analysis of a dilution series of a standard homogenate (preferably containing at least 3 mg/ml latex rubber) with the low latex sample homogenate in question.

It was also apparent that, when extraction buffer, instead of low latex content homogenate, was used to dilute the standard homogenate, quantification became less accurate at dilutions of 60% and below (Fig. 5, (△)). Extrapolation back to the starting material suggests that the amount of shrub should exceed a starting ratio of shrub to extraction buffer of 1:5.3 (w:v) to maintain maximum reproducibility in the latex quantification method. This ratio includes the buffer that is used in both the first and second grinds, because the subsequent filtrates would be pooled before latex quantification. In the Waring blender extraction method we described in Section 2.2.1, the pooled first two grinds reflect a shrub to buffer ratio of 1:4 (w:v).

3.3. Latex purity

Samples of three guayule branches sizes were ground 2×1 min and the latex was quantified, using the 1-ml method, in filtered homogenates from each grind and after the grinds were pooled. The purity of the coagulated latex was then determined (Table 5) as described in Section 2.5. The latex fraction was found to contain a solids component that appeared to increase with grinding and with the age of the tissue ground. Nonetheless, meaningful latex quantification data can still be obtained when not corrected for the solids component, a procedure which would greatly increase the time required for routine sample analysis (see Section 2.5). However, it is important to adjust for the solids contamination when calculating the total rubber balance of the sample (see Section 3.4).

To further test the accuracy of the 1-ml latex quantification method, the latex content of guayule branches of three branch sizes was analyzed in the filtered homogenate, pooled after 2×1 min grinds, as described in Section 2.2.1. Samples of the original branch material and of the bagasse after each filtration step, were analyzed for total rubber content, as described in Section 2.6. A materials balance was calculated, comparing the extractable latex and the rubber remaining in the bagasse with the total rubber in the branches before grinding and latex extraction (Table 6).

The empirical data in Table 6 reflect the latex content (column B, without adjustment for solids contamination of the coagulated latex) and rubber content in the bagasse calculated as a percentage of the dry weight of the bagasse used for the extraction (column C). By using these data sets to calculate the total rubber, the values (column B + C) obtained appear to overestimate the total rubber content of the original shrub (column A). If the two values agreed, the 'extraction discrepancy' numbers in column (B + C)/A would equal 1. The major part of the discrepancy between the rubber totals is caused by a flaw in the

Table 3
Effect of grinding on total rubber content of guayule branches of three different sizes[a]

Batch	No. of 1-min grinds	Branch diameter (cm)		
		≤0.5	0.5–1.0	>1.0
		(mg/ml)		
1	1	10.58	10.84	9.48
	2	11.55	11.08	9.14
	3	10.68	11.30	9.15
	4	10.74	10.42	9.99
Mean ± S.E.		10.89 ± 0.23	10.91 ± 0.19	9.44 ± 0.20
2	1	7.51	8.00	9.77
	2	7.23	8.12	9.69
	3	6.51	8.22	9.01
	4	6.26	8.04	8.26
Mean ± S.E.		6.88 ± 0.30	8.10 ± 0.05	9.18 ± 0.35

[a] The homogenate was filtered through a 1-mm steel mesh after each grind and the bagasse was then reground with fresh buffer. Values are the sum of extractable latex, determined by the 1-ml quantification method (see Section 2.3.1) and the residual rubber in the bagasse, determined by solvent extraction (Black et al., 1983) and corrected for the bagasse weight loss caused by grinding (see Section 3.4).

Table 4
Dry weight of latex extracted after one or two 1-min grinds of guayule branches of three different diameters[a]

Branch diameter (cm)	Grind No.		Pooled grinds	
	1	2	Calculated	Actual
	(mg/ml)			
≤0.5	12.25 ± 0.16 (8)	4.90 ± 0.08 (8)	8.58 ± 0.13	8.54 ± 0.10 (4)
0.5–1.0	17.12 ± 0.24 (8)	3.95 ± 0.35 (8)	10.54 ± 0.30	10.95 ± 0.06 (4)
>1.0	11.69 ± 0.24 (8)	2.12 ± 0.23 (8)	6.90 ± 0.24	6.47 ± 0.21 (4)

[a] The homogenate was filtered through a 1-mm steel mesh after the first grind and the bagasse was then reground with fresh buffer. Calculated pooled values are half the sum of the latex obtained from the first and second grinds. Actual pooled values were determined by mixing the homogenates from the first and second grinds prior to latex quantification. Each value is the mean ± S.E., with the number in each mean in parentheses.

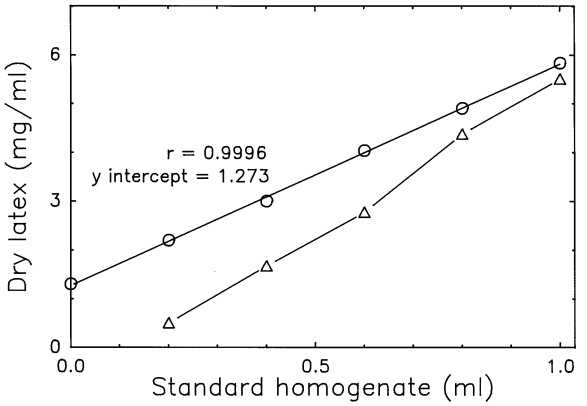

Fig. 5. Extractable latex quantified in a standard homogenate diluted with low latex homogenate (○) or with extraction buffer (△). Each value is the mean of 3 ± S.E.

Table 5
Percentage solids contamination (hexane insoluble) of coagulated latex from 1 ml quantification method[a]

Branch diameter (cm)	Grind No.		Pooled grinds
	1	2	
	(% solids)		
≤0.5	6.8 ± 0.7 (8)	7.8 ± 0.5 (8)	13.4 ± 0.9 (4)
0.5–1.0	6.0 ± 0.9 (8)	12.6 ± 2.6 (8)	12.0 ± 0.5 (4)
>1.0	13.6 ± 3.5 (8)	20.1 ± 2.7 (8)	14.9 ± 1.4 (4)

[a] Each value is the mean ± S.E. with the number in each mean in parenthesis.

bagasse rubber calculation (column C). For a tissue balance, the bagasse rubber must be calculated on the basis of the original weight of branch material and not expressed on the basis of the actual weight of bagasse that was solvent extracted to determine the rubber content. This is because the residual bagasse analyzed only consists of material >1 mm caught by the steel mesh filter. Although this bagasse fraction still retains the solid (non-latex) rubber component, it has lost the <1 mm component formed by grinding, including the soluble components that contribute to the dry weight of the intact branches. Thus, the weight used to calculate the percentage of rubber in the bagasse is too low and will result in a percentage rubber that is too high. Correction for this material to generate the true dry weight is essential because the material losses are large and vary depending upon the branch diameter (Fig. 6). As expected, the young tender branches have the greatest loss of material, whereas the mature branches with their large woody cores have the least (Fig. 6). On a dry weight basis, the average losses, after 2 × 1 min grinds, were: branches of <0.5 cm diameter = 38%, branches of 0.5–1.0 cm = 33% and branches >1.0 cm = 27% of their dry weight relative to the original branches. Thus, the true dry weight of the bagasse = 1/(fraction of bagasse remaining after filtering). The adjusted values 'rubber in bagasse' (column E of Table 6) were obtained by dividing the amount of solvent extracted rubber in the bagasse by the true dry weight of the bagasse.

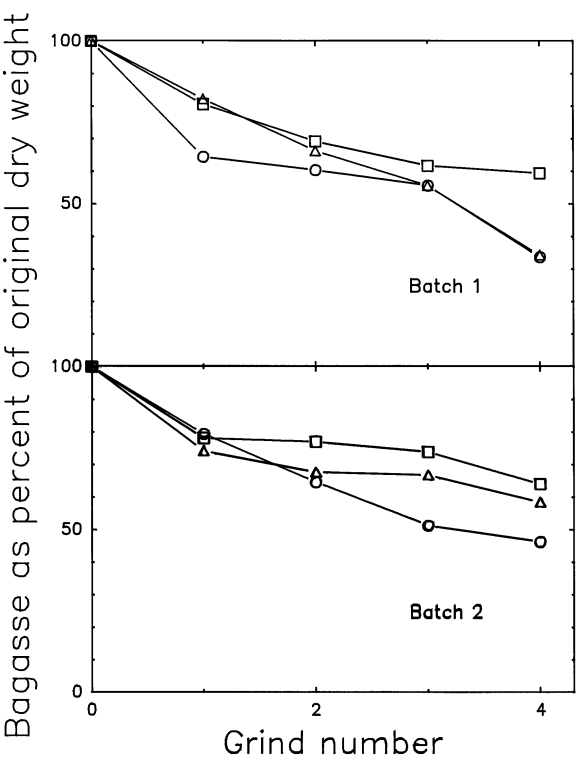

Fig. 6. Loss of bagasse dry weight as a function of number of grinds in two batches of guayule branches of three sizes. After each grind, the homogenate was filtered through a 1-mm steel mesh and the bagasse then reground with fresh buffer. Branch diameters were (A) ○, ≤0.5 cm; (B) △, 0.5–1.0 cm; (C) □, >1.0 cm.

Table 6
Rubber and latex content of guayule branches of three different diameters, including comparison of the total amount of rubber quantified before and after latex extraction, expressed as percentages of dry weight[a]

Batch	Branch diameter (cm)	Total rubber in branch (%)	Empirical				Adjusted				Rubber in extractable latex form (%)
			Latex (%)	Rubber in bagasse (%)	Sum (%)	Extraction discrepancy	Latex (%)	Rubber in bagasse (%)	Sum (%)	Extraction discrepancy	
		A	B	C	B+C	(B+C)/A	D	E	D+E	(D+E)/A	(D/A)100
1	≤0.5	9.37	8.32	5.37	13.69	1.46	7.73	3.23	10.96	1.17	82
	0.5–1.0	9.89	8.69	3.62	12.31	1.24	8.16	2.39	10.55	1.07	83
	>1.0	5.81	7.24	2.74	9.98	1.72	6.18	1.89	8.07	1.39	106
2	≤0.5	6.81	4.55	4.15	8.70	1.28	4.23	2.68	6.91	1.01	62
	0.5-1.0	7.89	6.05	3.06	9.11	1.15	5.68	2.07	7.75	0.98	72
	>1.0	6.37	4.57	6.66	11.23	1.76	3.90	5.12	9.02	1.42	61

[a] Rubber in branches and bagasse was determined using solvent extraction (Black et al., 1983). Empirical latex values were determined after extraction of the latex using 2×1 min grinds (see Section 2.2.1) followed by quantification of the weight of coagulated latex using the 1-ml method (see Section 2.3.1). Adjusted latex values have had the solids component subtracted (see Table 5). Empirical bagasse values were calculated based upon the dry weight of the bagasse, whereas adjusted values were calculated to reflect the equivalent original branch weight of the bagasse (see Fig. 6).

Table 7
Analysis of different guayule homogenates using the 1-ml latex quantification method[a]

Branch diameter (cm)	Grind No.	Waring homogenate		Oster homogenate	
		Lab 1	Lab 2	Lab 1	Lab 2
		mg/cm^3			
≤0.5	1	6.46	6.67	2.32	2.84
		6.55	6.77	1.76	2.68
		6.63	6.45	1.82	2.39
	2	2.20	2.09	0.08	0.32
		1.76	1.36	0.21	0.25
		1.95	1.37	0.02	0.19
0.5–1.0	1	11.30	12.93	3.77	5.52
		11.90	13.83	3.98	4.40
		12.15	13.19	4.11	4.67
	2	3.91	6.57	0.49	1.52
		3.68	6.72	0.44	1.49
		3.40	4.43	0.87	1.57
>1.0	1	11.72	13.58	4.18	5.40
		11.48	17.37	4.11	5.31
		11.44	13.46	4.40	5.32
	2	4.32	8.04	1.20	1.62
		4.58	7.95	0.62	1.58
		4.16	7.14	0.49	1.73

[a] Both homogenates reflect the same fresh shrub weight:extraction buffer ratio (1:4 w:v).

After correction for bagasse weight and for solids contamination of the coagulated latex, the sum of the extracted latex rubber and the rubber remaining in the bagasse after 2×1 min grinds (as in the recommended method for latex quantification) agreed well with the total rubber extracted from the original branches of <0.5 and 0.5–1.0 cm in diameter (Table 6, adjusted values). A discrepancy still occurred in branches >1.0 cm in diameter. However, we suggest that this discrepancy actually reflects incomplete solvent extraction of these large branches, especially as the total rubber calculated over four grinds (Table 3, which includes the bagasse correction described in this section) did not change with grinding number.

The data in the table also indicated that most of the rubber in the guayule branches is still in the form of extractable latex (see final column, Table 6). The lower values obtained for the 'batch 2' branches may be due to inter-batch variability or to the longer post-harvest storage period experienced by this material compared with 'batch 1'. For example, during storage, some of the free rubber particles—that constitute the latex rubber fraction—may have coagulated in situ.

Nonetheless, consistent differences between the different branch sizes are apparent. Branches of 0.5–1.0 cm in diameter contain the greatest percentage of latex (Tables 2 and 6).

3.5. Comparison and inter-laboratory reproducibility of latex quantification methods

The reproducibility of the two latex quantification methods was tested by their use by researchers at two locations. Homogenates were prepared by both extraction methods using two grinds (2×1 min grinds with the Waring blender, as described in Section 2.2.1 and a 2 min followed by a 1 min grind with the Oster

blender, as described in Section 2.2.2), shipped overnight on ice to the other research group and analyzed at the same time the next day, using the 1- and 14-ml quantification methods. The results are shown in Tables 7 and 8. We used a structural relation model to compare the data produced by the two quantification methods, because such a model estimates an equation relating two variables that both contain measurement error (Fig. 7). All three plant sizes and both grinds were used to examine the relationship across a wide range of values. The square root transformation helped stabilize the variance across the range of measurement. It is clear that both the 1- and 14-ml latex quantification methods gave similar results, independent of the extraction method or the branch diameter analyzed and could be used effectively by both laboratories. We also found the latex drying temperature (37°C in the 1-ml method and 60°C in the 14-ml method) gave equivalent results (data not shown).

3.6. Comparison of different extraction methods

The latex content of homogenates produced by the two different extraction methods (Section 2.2.1 and Section 2.2.2) was also compared, using both 1- and 14-ml latex quantification methods (Section 2.3.1 and Section 2.3.2). These data clearly show that higher latex values were consistently obtained from the Waring homogenate than the Oster homogenate (Tables 7 and 8). When two grinds were used, the Waring blender method (2×1 min grinds) yielded 2.3–4.1 times the latex produced by the Oster blender method (a 2-min followed by a 1-min grind) (Table 9) independent of the quantification method used. A structural relation model demonstrated substantial underestimation of the amount of latex extracted using the Oster blender, as described by the regression equation (Fig. 8), consisting of a constant deficit (the y intercept) plus a constant proportion of any additional latex extracted (the slope). However, because the experimental data fit

Table 8
Analysis of different guayule homogenates using the 14-ml latex quantification method[a]

Branch diameter (cm)	Grind No.	Waring homogenate	Oster homogenate	
		Lab 1	Lab 1	Lab 2
		mg/cm^3		
≤0.5	1	5.55	2.41	3.73
		5.86	1.89	3.37
		6.18	1.37	3.78
	2	0.55	0.42	0.80
		1.44	0.12	0.78
		1.34	0.50	0.58
0.5–1.0	1	10.04	5.65	6.68
		10.60	4.77	6.90
		10.20	4.73	5.21
	2	4.04	0.89	1.40
		3.58	0.39	1.42
		2.14	0.89	1.18
>1.0	1	10.68	4.05	5.09
		10.65	4.23	4.55
		11.54	5.26	5.18
	2	3.41	0.21	1.23
		3.69	0.28	1.32
		3.59	0.36	1.23

[a] Both homogenates reflect the same fresh shrub weight:extraction buffer ratio (1:4 w:v).

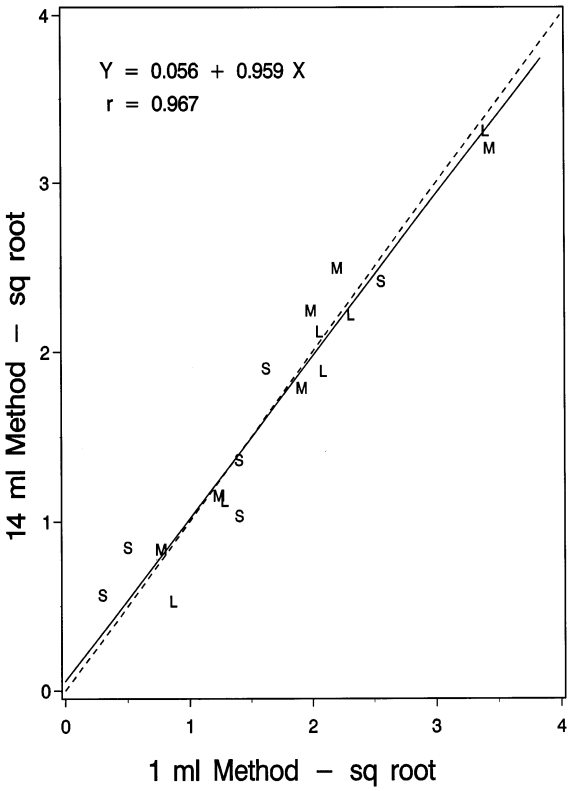

Fig. 7. Comparison of the 1- and 14-ml latex quantification methods using a structural relation model. The solid line shows the structural relationship estimated from the data and the dashed line, the expected line if both methods yield precisely the same results. Symbols refer to branch diameters analyzed: S, ≤ 0.5 cm; M, 0.5–1.0 cm; L, >1.0 cm. Each value is the mean of three determinations.

a linear regression, latex yield appeared independent of the quantification method or type of tissue (Fig. 8). Thus, it is apparent that the homogenization method can introduce large differences in the apparent amount of extractable latex. The discrepancy was caused by the slower blade rotation of the Oster (18 000 rpm) compared with the Waring (22 000 rpm) blender. When the amount of latex released with Oster blender grind time was analyzed (as was done for the Waring blender, see Figs. 2 and 3) to optimize the extraction procedure, we found that increasing Oster grind times to 4 and 3 min, respectively, adequately extracted the latex from the guayule shrub (data not shown).

3.7. Inter-laboratory latex quantification reproducibility

Although the two quantification methods gave the same results when performed by the same researchers (Fig. 7), we did notice that one laboratory (Lab. 2) consistently estimated slightly higher latex quantities than the other (Tables 7 and 8). Structural analysis showed that this variation was consistent over all branch sizes and extraction methods (Fig. 9). Thus, Lab. 2 consistently estimated 0.104 mg/ml more latex in homogenates than Lab. 1 over all concentrations and branch diameters (with confidence limits of 0.0089 and 0.3035). This comparison provides an indication of the variation that is to be expected among different laboratories.

4. Discussion

It is clear that effective latex quantification contains two distinct components: the extraction method and the quantification method. Both methods must be reproducible and independent of any particular experimenter. In this study, the variation introduced by the different extraction methods was much larger than that caused by the different quantification methods, or by the different researchers employing those methods. Thus, grinding protocols must be optimized so that they directly relate to the maximum possible amount of latex extractable from the shrub. As demonstrated in this paper (Fig. 8, Table 9), large errors in apparent latex content can be introduced by insufficient tissue disruption during grinding. Also, meaningful information, from a commercialization point of view, should allow extrapolation to the amount of latex that would be obtained if it were all released from the shrub during grinding. A commercial processing plant, undoubtedly, will be based upon the best available, large scale extraction methods, which may improve upon the best achievable in a research laboratory. In this paper, we have used a combination of visual assessment, particle size analysis and latex quantification to optimize the Waring blender extraction method described in Section

2.2.1. Such methods could be readily applied to any other wet-grinding extraction technique to optimize grinding time. In our study, the particle size analysis of the homogenate (Fig. 1) agreed well with the latex quantification data (Fig. 2; see also Figs. 3 and 4 and Tables 1 and 2), both indicating that 2 min of grinding (2 × 1 min), with a Waring blender, is sufficient to release at least 90% of the extractable latex from the guayule shrub. Although additional grinds achieve slight additional latex yield (Fig. 3, Table 1), they may not be cost-effective for a commercial-scale process (Cornish, 1996).

The two latex quantification methods described in this paper (Section 2.2.1 and Section 2.2.2), measure the rubber particles released during tissue homogenization into aqueous suspension and are, therefore, directly applicable to the proposed commercial latex extraction and purification process (Cornish, 1996). Both quantification methods produce comparable data and can be used effectively on three different sizes of guayule branches and in the presence or absence of leaves. Either variation could be employed to test the efficiency of processing steps during the construction and optimization of a large-scale processing plant. However, the 1-ml quantification method is both simpler and faster than the 14-ml method (60 samples in 1 h, compared with 36 samples in 5 h). The 1-ml method can be used on much smaller samples than the 14-ml method and should prove useful for screening young plants, or parts of guayule shrub. However, the larger volume in the 14-ml method allows homogenates with lower latex concentrations (down to 0.3 mg/ml) to be accurately quantified, whereas 2 mg/ml is the lower limit for easy use of the 1-ml method. Of course, the more time-consuming adaption of the 1-ml method described (Section 3.2) can be easily used to accurately quantify latex in materials with unusually low latex contents.

Nonetheless, homogenate preparation is the primary limiting factor as far as number of samples processed is concerned. With one Waring blender, three samples of 50–200 g shrub can be prepared for quantification in 1 h, compared with two samples with the Oster blender. Although multiple blenders would considerably increase sample numbers, it is clear that comparably efficient extraction methods, whether for several plants together or parts of single plants, must be used by different researchers. Without optimized methods, comparisons of different data sets of extractable latex content will prove meaningless.

Our data show that latex can be readily quantified in a range of guayule branch sizes and homogenates, allowing detailed studies on the optimization of agronomic parameters, conditions for guayule harvest, storage post-harvest and storage of homogenates at different steps of the latex extraction process. However, as data are generated by many researchers and hypoallergenic

Table 9
Comparison of the two latex extraction methods[a]

Quantification method	Branch diameter (cm)	Waring homogenate		Oster homogenate		Waring/Oster	
		Lab 1	Lab 2	Lab 1	Lab 2	Lab 1	Lab 2
		mg/cm^3 –					
1 cm^3	≤0.5	4.26	4.12	1.03	1.45	4.14	2.84
	0.5–1.0	7.73	9.61	2.28	3.20	3.39	3.00
	>1.0	7.95	11.26	2.50	3.50	3.18	3.22
14 cm^3	≤0.5	3.49	–	1.12	2.18	3.12	–
	0.5–1.0	6.77	–	2.89	3.80	2.34	–
	>1.0	7.26	–	2.40	3.10	3.03	–

[a] Homogenates were analyzed using both latex quantification methods. Values are means pooled from three determinations of latex content of homogenates from two grinds (2 × 1 min). The pooled homogenates reflect a 1:4 fresh shrub weight:extraction buffer ratio.

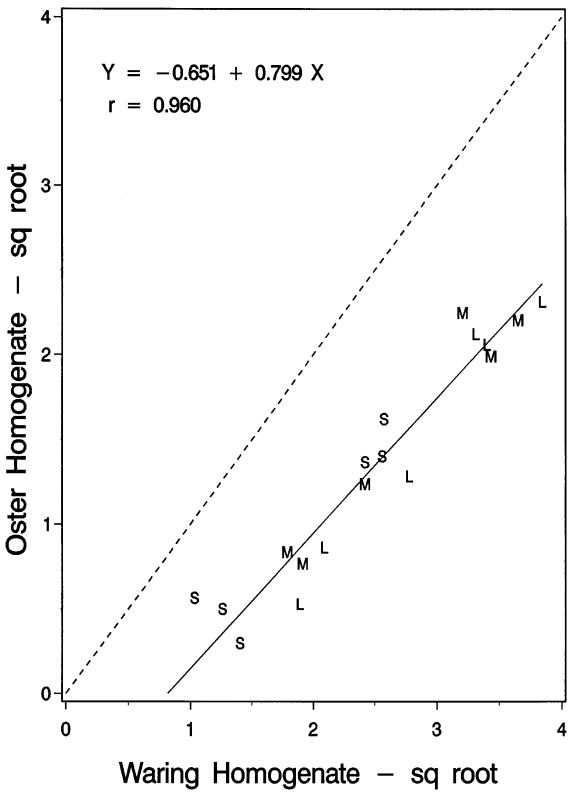

Fig. 8. Comparison of the Waring and Oster extraction methods, after using 2×1 min grind times, with a structural relation model. The solid line shows the structural relationship estimated from the data and the dashed line the expected line if both methods yield precisely the same results. Symbols refer to branch diameters analyzed: S, ≤ 0.5 cm, M, 0.5–1.0 cm; L > 1.0 cm. Each value is the mean of three determinations.

guayule latex commercialization becomes a reality, it is vital that data are presented in a useful, standardized format. With respect to guayule plants, this format must be directly relatable to latex yield per plant and per hectare. To explain this point further, it is important to consider where, when and how rubber is made in guayule, as well as guayule's annual growth habit. Rubber is made primarily in the winter months, but a low rate of synthesis continues throughout the rest of the year (Cornish and Siler, 1996b). Also, rubber is an end product stored in rubber particles in the bark parenchyma. Rubber yield per plant never declines because rubber is not degraded in living guayule. Latex yield may differ at different times of the year and is one of the parameters currently under investigation. However, if rubber or latex yield is expressed on a plant weight basis, a misleading picture can emerge. For example, guayule plants have more leaves in summer than in winter and because rubber content is only slowly increasing at this time, rubber (and latex) per gram would appear to decline as the leaf canopy increases. Yield per hectare, however, is undoubtedly increasing! Also, rubber and latex yield will be much more comparable if expressed on a dry weight basis. A fresh weight basis would lead spuriously to apparent decreases in latex and rubber content after rain and increases during drought or after a wind storm. Of course, none of

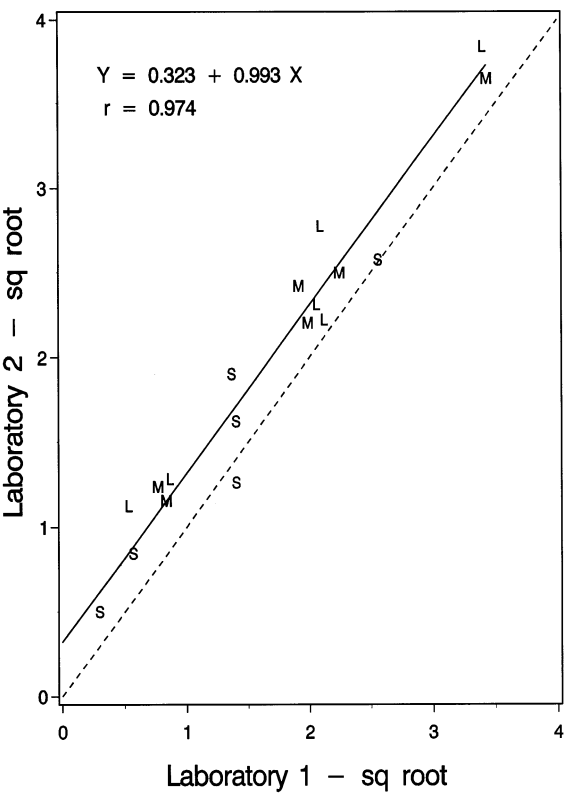

Fig. 9. Comparison of latex quantification as performed by two research laboratories, using a structural relation model. The solid line shows the structural relationship estimated from the data and the dashed line the expected line if both methods yield precisely the same results. Symbols refer to branch diameters analyzed: S, ≤ 0.5 cm; M, 0.5–1.0 cm; L, > 1.0 cm. Each value is the mean of three determinations.

these transient changes in the concentration of rubber in the tissue, or the relative proportion of rubber-containing and non-rubber-containing tissue on a plant, have any effect on yield per hectare on the commercial farm.

5. Conclusion

Our results show that the amount of latex in guayule shrub can be accurately quantified by either of two variations of a simple method, although the 1-ml method is simpler and faster than the 14-ml method and can be used on smaller samples. Homogenate preparation is critical to successful latex quantification and appropriate extraction times must be established for each blender to be used. Using the Waring blender, we have shown that at least 90% of the endogenous latex can be released by two 1-min grinds and at least 99% by four grinds. The proportion of latex rubber, compared with the total amount of rubber in guayule branches, varied from 60 to 100%, depending upon branch diameter and the post-harvest history of the shrub. Branches between 0.5 and 1 cm in diameter consistently had a higher latex content, on a dry weight basis, than either smaller or larger branches.

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