

A Six Step Harvesting Procedure for Guayule Small Plots for Laboratory Analyses

T.A. Coffelt* and F.S. Nakayama

Hevea [*Hevea brasiliensis* (A. Juss.) Muell.-Arg., Euphorbiaceae] latex allergies in the United States have become a serious health problem in certain population groups, especially health care workers and patients who undergo multiple surgeries (Ownby et al. 1994). Guayule (*Parthenium argentatum* Gray, Asteraceae) has been demonstrated to be a source of hypoallergenic latex to replace *Hevea* latex (Siler and Cornish 1994). Guayule latex has been shown to prevent transmission of viruses and other pathogens, making it suitable for use in medical products, gloves, and condoms (Cornish et al. 1996). In addition, guayule can be grown in the United States to provide a domestic source of natural latex and rubber (Thompson and Ray 1988). The United States imports more than one million tonnes of natural rubber at a cost of about one billion US dollars (Mooibroek and Cornish 2000).

Several improved guayule germplasm lines have been released for use in breeding programs and cultivar development (Estilai 1986; Ray et al. 1999). Breeding programs in the past have focused on screening germplasm and developing improved lines with higher rubber and/or resin contents. Thus, previous harvesting protocols have not had to deal with the problem of latex loss as plant material is being processed. A new protocol that allows harvesting of various amounts of plant material in a manner that minimizes latex loss is needed by guayule researchers. A standardized protocol will also allow better comparison of research results from various areas of the world where guayule research is being conducted. The objective of this study was to develop a protocol that could be used to harvest variable amounts of plant material from agronomic and breeding tests for laboratory analyses.

MATERIALS AND METHODS

Equipment

The equipment used for harvesting plants depended upon the number of plants to be harvested at one time. A pair of hand held lopping shears was used when less than 30 plants were harvested in one day. An electric reciprocating saw (Model 3103, Black and Decker, Inc., Hunt Valley, Missouri) was used when more than 30 plants were harvested in one day. The blades for the saw were a pruning type blade 241 mm long with a spacing of 42 mm between teeth. A portable generator on wheels provided electricity for the saw.

Plants were chipped using a Troy-Bilt Model 47321 Chipper/Shredder (Garden Way, Inc., Troy, New York). The chipper was modified in two ways (Fig. 1, 2). The first modification was placing hinges on the front panel and replacing the bolts with quick disconnect pins. This modification was made to allow easier and complete cleaning of the interior of the chipper to prevent mixing between samples. The second modification was to replace the wheels of the chipper with four adjustable legs. This modification allowed the chipper/shredder to be raised high enough to insert a collection tub below the discharge area of the chipper/shredder. A 9.53 mm, round-holed screen (Fig. 3) was used in the chipper to size chipped material before it passed through to the collection tub.

The collection tub used was 540 mm long × 380 mm wide, and 300 mm deep. The opening of the collection tub matched the discharge opening of the chipper so that minimal amounts of chipped material were lost during chipping. The collection tub was large enough to hold the amount of material being chipped from a 10 kg sample. The tub was made of latex free material to prevent sample contamination.

*Mention of equipment trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by USDA-ARS to the exclusion of other products of similar nature.

The authors thank Greg Leake and Stephen Vinyard, for technical assistance

Proposed Protocol

The protocol developed consisted of six steps. Step one was to cut the plants in the field as close as possible to ground level (50 mm). Step 2 was to transport the plants from the field to the chipping area as soon as possible in bags that kept plant samples separate. Step 3 was to obtain a fresh weight for each sample. Step 4 was to process the sample through the chipper and obtain a fresh weight of the chipped material. After weighing and prior to chipping the bag(s) containing the plant material to be chipped was emptied onto a plastic sheet to minimize sample loss during the chipping process. After the larger plant parts had been chipped, the plastic sheet was carefully picked up and the smaller plant materials remaining on the plastic sheet were dumped into the chipper/shredder. Step 5 was to add antioxidant solution (0.2% sodium sulfite in distilled water at a pH of about 11) so that fresh weight of plant material collected and antioxidant solution are in a 1:1 ratio. Step 6 was to thoroughly mix the antioxidant solution with the plant material by stirring the plant material after the antioxidant solution was added. Steps 2–6 were done in less than 3 hr to minimize latex loss. The mixture was stored at 4 to 10°C prior to laboratory analysis for latex using the method developed by Cornish et al. (1999). While this mixture can be stored under these conditions up to five weeks without loss of latex (Cornish et al. 2003b), samples used in the studies discussed in this paper were processed within 3–5 days after harvest.

Measurements and Evaluations

The proposed protocol was evaluated by processing samples harvested using this protocol for latex content. If samples harvested using the proposed protocol could be processed for latex using the recommended laboratory protocol of Cornish et al. (1999) without additional preparation prior to using the laboratory protocol, then we considered the proposed harvesting protocol acceptable. Additionally, the proposed protocol should provide samples that could be used for rubber, resin, and other laboratory analyses. Other desirable performance characteristics of the proposed harvesting protocol were that at least eight samples or plots can be processed in an hour or less with sample purity being maintained.

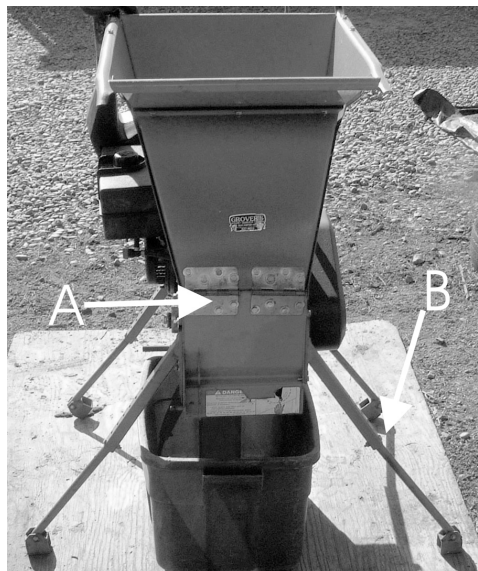


Fig. 1. Chipper/shredder used to harvest guayule research plots. A. Hinges added to open inside of chipper/shredder for easier cleaning of the interior of the chipper/shredder between plots. B. Legs added to raise the chipper/shredder high enough to accommodate a collection tub.

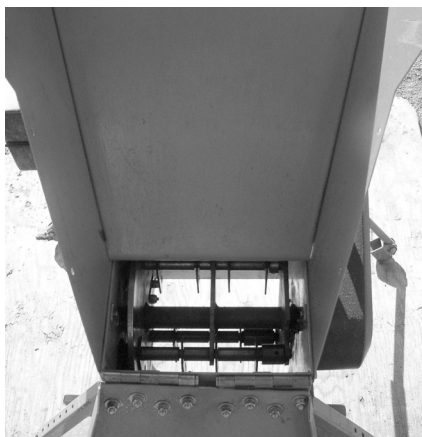


Fig. 2. Chipper/shredder in open position for clean out of the interior of the chipper/shredder between guayule samples.

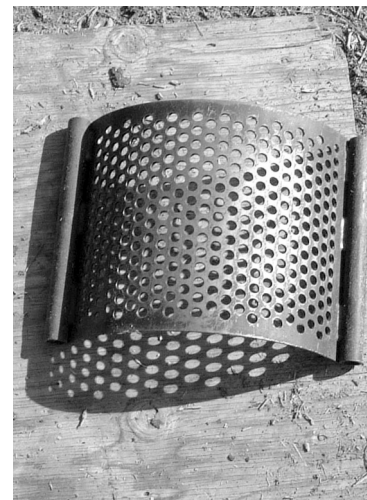


Fig. 3. The 9.53 mm round holed screen used in the chipper/shredder to size chipped guayule plant material passing through to the collection tub.

An experiment with three replications (runs) was conducted using the breeding line CAL-7 to determine the effects of delaying addition of the antioxidant solution to the chipped material. In the first run, plants were chipped and five sub-samples taken. Antioxidant solution was added immediately to the first sub-sample (time 0), then at 8 min to the second sub-sample, 24 min to the third sub-sample, 36 min to the fourth sub-sample, and 70 min to the fifth sub-sample after chipping. In the second run five sub-samples were taken from freshly chipped plant material and the times for adding antioxidant solution were 0, 5, 12, 30, and 60 min after chipping. In the third run five sub-samples were taken from freshly chipped plant material and the times for adding antioxidant solution were 0, 5, 17, 36, and 80 minutes after chipping. All samples were evaluated for percentage latex using the method of Cornish et al. (1999).

RESULTS AND DISCUSSION

Observations

The first step in the harvesting protocol (cutting the plant material in the field) has been done successfully using either the hand lopping shears or an electric reciprocating saw. For small samples of less than 30 whole plants, the lopping shears worked satisfactorily. However, for larger numbers of samples and plants the electric saw is faster and easier to use. The electric saw has been used to harvest up to 160 plants in an hour. The electric saw provided a more uniform stump following harvest than the lopping shears. Thus, if regrowth measurements and evaluations are planned, the electric saw may be more desirable even if less than 30 plants are harvested.

The second step of transporting the cut material to the chipping location requires that the material be moved soon after cutting to minimize latex loss and maintain the sample integrity. We have found that plastic garbage bags, plastic mesh bags, or burlap bags can be used successfully. Twenty samples of about 4 kg or less per sample can be harvested and moved a short distance (less than 45 min) prior to starting the chipping process without latex loss (F.S. Nakayama and T.A. Coffelt, unpubl. results, 1998). However, for larger samples or a greater number of samples, samples should be moved and the chipping process begun by a separate crew while the harvesting continues or harvesting the plants should be done in stages to insure that samples are chipped in a timely manner.

The third step was done at the chipping location. Samples were weighed to obtain a fresh weight for use in determining yields of biomass, rubber, latex, and/or resin. Care should be taken to minimize loss of plant material between the weighing and chipping steps. Any large capacity platform type electronic balance is acceptable for obtaining fresh weights. The major requirement is that the balance has enough capacity to weigh the plant material being harvested and the container holding the plant material.

In the fourth step, the first modification of the chipper (Fig. 1A) allowed access to the interior of the chipper (Fig. 2) for quick clean out between plots, thus preventing cross contamination of samples. The second modification (Fig. 1B) allowed the chipper to be raised for easy insertion of a collection tub below the chipper. The screen in the bottom of the chipper (Fig. 3) was easily removed for cleaning between samples and plots by removing two pins and rods. The chipper performed well using samples from 1 kg to over 40 kg in size. Under the growing conditions in Arizona, small plants less than 0.5 kg did not chip well and resulted in clogging of the screen. If chipping of small plants is required, modifications would need to be made to the recommended protocol by changing the screen opening size.

The fifth step of adding antioxidant can be done as a one step process as described above or as two steps. If done as two steps, an initial amount of antioxidant solution (0.2% sodium sulfite in distilled water at a pH of about 11) is added to the collection container and additional solution is added following chipping to make the total solution by weight equal to the chipped plant material weight. The two step process works with smaller samples and when large numbers of samples are not being processed. The advantage to the two step process is that the antioxidant solution comes into contact with the chipped material sooner, thus reducing the loss of latex. As long as the delay from chipping to addition of the antioxidant solution is less than 3 min (Fig. 4), the proposed one step process of the protocol is acceptable.

During the fifth step, the whole sample can be collected for mixing with the antioxidant solution or a smaller container can be placed inside the collection tub to collect a sub-sample. Two smaller size containers

have been used satisfactorily to collect a sub-sample. A 1.8 L container will hold about a 200 to 700 g sample, while a 9.5 L container can hold up to 3,000 g. The advantage to the smaller sample is the use of less antioxidant solution and shorter harvesting times. A sample size of about 250 g (plant material plus antioxidant) is sufficient for latex analyses by the proposed method of Cornish et al. (1999). A larger sample may be needed if additional analyses such as rubber and resin contents (Black et al. 1983), and/or latex quality (Cornish et al. 2003a) are planned. When plant samples > 2 kg are processed, it may be necessary to place a screen over the sub-sample collection container so that the sub-sample is representative of the whole sample chipped and not only the plant material chipped first.

The last step is critical. The sample and antioxidant solution need to be thoroughly mixed to prevent the loss of latex. Teetor et al. (2003) have shown that the concentration of various components such as rubber, resins, and guayulins can vary with plant part. Since plant parts are not always mixed in equal proportions during chipping, the sample should be thoroughly mixed prior to sampling for laboratory analyses. A well-mixed sample will insure that the results are representative of the whole plant. Once the plant material and solution have been thoroughly mixed, the sample can be analyzed in the laboratory by standard procedures for latex, rubber, and resin concentration or stored under controlled temperature conditions (4 to 10°C). Results from other studies have shown that once the chipped material is processed to the homogenate stage it can be stored for up to five weeks prior to completing the analyses without loss of latex (Cornish et al. 2003b).

Evaluation of Protocol

Guayule stores its latex (rubber) within cells of the bark and woody tissues of the plant, unlike *Hevea brasiliensis* which stores its latex in a system of ducts that can be tapped (Wagner and Schloman 1991). In order to extract the latex from guayule the latex containing cells must be ruptured. The latex can then be extracted from the plant material with water (Cornish et al. 1999) and later concentrated by separating the latex from the water. This extraction process allows the latex to remain in a fluid state rather than being converted irreversibly to a solid rubber state. Steps 4 to 6 of the proposed protocol begin this extraction process, which is then completed in the laboratory by the method of Cornish et al. (1999). The antioxidant solution is used in place of water to minimize degradation of the latex by microorganisms before the extraction process is completed.

Results (Fig. 4) from the experiment to determine the effects of delaying addition of the antioxidant solution to the chipped material show that the loss of latex occurs rapidly. Losses of 15% (Run 2) to 31% (Run 3) of the total extractable latex (Time 0) occurred within 5 min without addition of the antioxidant solution. Almost 70% of the extractable latex was lost within 60 min after chipping without the addition of antioxidant solution. These results show the importance of adding the antioxidant solution immediately after chipping if a procedure is not available to add the solution during chipping. While addition of the solution during chipping may be feasible on a commercial scale, it is currently not practical for chipping of small research plots.

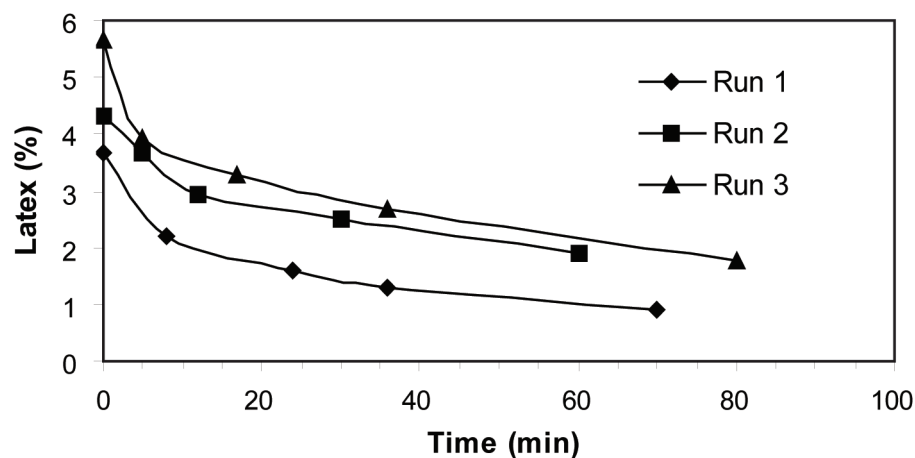


Fig. 4. Decrease in percentage of guayule latex extraction with time for three runs with time to addition of antioxidant solution varying from 0 to 80 min.

Steps in the proposed protocol are similar on a small scale to those proposed by Coates (1991) for harvesting fields of guayule for rubber extraction and methods used previously to harvest small plots for rubber and resin analyses (D.T. Ray, pers. commun., 2003). The major difference between harvesting for rubber and harvesting for latex is that optimal latex extraction necessitates the addition of the antioxidant solution immediately after chipping. The proposed protocol ensures more timely processing of plant material than is required for solid rubber extraction. The proposed protocol also specifies a screen size that plant material must pass through. Previous protocols for harvesting guayule for rubber have used various types of chippers with and without screens for sizing the chipped plant material. Chippers that result in large pieces of plant material (greater than that using the 9.53 mm round-hole screen) do not result in efficient latex extraction and require further chipping prior to laboratory analyses.

This basic protocol has been used for over five years to successfully analyze guayule plant material from plants of various ages and sizes (Table 1) for latex, rubber, and resin concentration (Coffelt et al. 2000, 2003, 2005; Dierig et al. 2001; Nakayama et al. 2002; Cornish et al. 2003a; Majeau et al. 2003; Veatch et al. 2003). The proposed protocol has been used successfully in these studies to harvest from less than 1 kg to over 20 kg of plant material from field plots of breeding and agronomic studies with a minimal loss of latex (Table 1).

Several workers (Coffelt et al. 2000, 2005; Dierig et al. 2001; Nakayama et al. 2002; Majeau et al. 2003) have used the protocol in breeding and agronomic studies. Coffelt et al. (2005) and Dierig et al. (2001) successfully used the protocol to determine genotype × environment effects both within and between locations on latex content of various guayule lines. The harvested plant sample size for chipping ranged from less than 1 kg to over 13 kg in these studies. Similar results (Table 1) have been obtained in other breeding and agronomic studies evaluating germplasm lines (Coffelt et al. 2000; Majeau et al. 2003), heritability (Dierig et al. 2001), harvesting dates (Nakayama et al. 2002), and post-cutting, pre-chipping storage effects on latex content (Coffelt et al. 2003). Sample sizes over 20 kg have been processed from a study of plant populations and planting dates (Cornish et al. 2003a), and the protocol has also been used successfully to evaluate transgenic plants for potentially increased latex production (Veatch et al. 2003). Coffelt et al. (2000) demonstrated that by using this protocol one could obtain information on fresh and dry biomass yields, latex concentration and yield, and potential losses during the chipping process. More recent studies have shown that the protocol has been used successfully to obtain rubber, resin, and guayulin A and B concentrations and yields (Coffelt et al. 2003; Majeau et al. 2003) and latex quality evaluations (Cornish et al. 2003a).

The successful use of the proposed protocol in the above studies demonstrates that the proposed protocol satisfies the objective of developing a protocol that can be used to harvest various amounts of plant material from agronomic and breeding tests for laboratory analyses of latex, rubber, resin, guayulins, and latex qual-

Table 1. Number of plots harvested, plots harvested/harvest date, number of plants harvested/plot, plant ages at harvest, range of plant fresh weights at harvest, range of sample weights collected for analyses, and range of latex concentrations in studies used to verify proposed protocol for harvesting guayule experimental plots for latex analyses.

Study	Total plots	Plots/harvest	Plants/plot	Plant age (yr)	Total plant fresh weight (kg)	Total plant sample weight (g)	Latex (%)
Germplasm	88	11	2	3	0.77–4.05	610–3,460	2.19–4.22
Heritability	40	12	2	2, 3	1.42–4.98	300–428	0.30–2.70
Genotype × environment	128	8	2	1, 2, 3	0.99–13.32	150–610	0.03–3.85
Planting date/spacing	288	32	8–22	1, 1.5, 2, 2.5, 3	1.41–20.69	264–2,132	0.11–2.97
Yield	96	24	2	1.5	0.67–6.51	229–1,384	0.37–3.64
Harvest date 1	216	8, 12	2	3–5	0.19–12.66	150–13,320	0.36–4.36
Harvest date 2	204	12	2	3–4	0.66–20.51	96–651	0.10–4.03

ity. The modifications to the chipper have also made it easy to maintain sample integrity by making cleaning of the chipper between plots easy. This protocol will be of use to other researchers around the world who are now beginning to conduct research on guayule and provide researchers with a common harvesting protocol to compare results better.

REFERENCES

- Black, L.T., G.E. Hamerstrand, F.S. Nakayama, and B.A. Rasnik. 1983. Gravimetric analysis for determining the resin and rubber content of guayule. *Rubber Chem. Technol.* 56:367–371.
- Coates, W. 1991. Guayule harvesting equipment. p. 241–259. In: J.W. Whitworth and E.E. Whitehead (eds.), *Guayule natural rubber*. Office of Arid Lands, Univ. Arizona, Tucson.
- Coffelt, T.A., D.A. Dierig, D.T. Ray, and F.S. Nakayama. 2000. Evaluation of guayule germplasm for agronomic and latex characteristics. *AAIC Annu. Meeting Prog. Abstracts.* p. 23.
- Coffelt, T.A., F.S. Nakayama, D.T. Ray, and K. Cornish. 2003. Post-harvest guayule storage tests. *AAIC Annu. Meeting Prog. Abstracts.* p. 25.
- Coffelt, T.A., D.T. Ray, F.S. Nakayama, and D.A. Dierig. 2005. Genotypic and environmental effects on guayule (*Parthenium argentatum*) latex and growth. *Indust. Crops Prod.* 22:95–99.
- Cornish, K., H.F. Bader, and C.D. Lytle. 1996. Manufacture and testing of guayule latex products. Paper presented at the 1996 Int. Meeting AAIC, 22–25 September 1996, San Antonio, TX.
- Cornish, K., M.H. Chapman, F.S. Nakayama, S.H. Vinyard, and L.C. Whitehand. 1999. Latex quantification in guayule shrub and homogenate. *Indust. Crops Prod.* 10:121–136.
- Cornish, K., R.G. McCoy III, J.L. Bricha, T.A. Coffelt, F.S. Nakayama, and D.T. Ray. 2003a. Quality of latex extracted and purified from guayule agronomic trials with different spacings and dates of planting. *AAIC Annu. Meeting Prog. Abstracts.* p. 12.
- Cornish, K., R.G. McCoy III, J.E. Van Fleet, J.L. Bricha, and B.E. Knuckles. 2003b. Latex yield and quality during storage of guayule (*Parthenium argentatum* Gray) homogenates. *AAIC Annu. Meeting Prog. Abstracts.* p. 26.
- Dierig, D.A., D.T. Ray, T.A. Coffelt, F.S. Nakayama, G.S. Leake, and G. Lorenz. 2001. Heritability of height, width, resin, rubber, and latex in guayule (*Parthenium argentatum*). *Indust. Crops Prod.* 13:229–238.
- Estilai, A. 1986. Registration of Cal-6 and Cal-7 guayule germplasm. *Crop Sci.* 26:1261–1262.
- Majeau, G.H., D.T. Ray, T.A. Coffelt, and M.A. Foster. 2003. New guayule (*Parthenium argentatum* Gray) variety trials. *AAIC Annu. Meeting Prog. Abstracts.* p. 14.
- Mooibroek, H. and K. Cornish. 2000. Alternative sources of natural rubber. *Appl. Microbiol. Biotechnol.* 53:355–365.
- Nakayama, F.S., T.A. Coffelt, S.H. Vinyard, G.S. Leake, and A.L. Faber. 2002. Frequency distribution analysis of bimonthly guayule latex yield for selecting optimal harvest dates. *AAIC Annu. Meeting Prog. Abstracts.* p. 7.
- Ownby, D.R., H.E. Ownby, J.A. McCullough, and A.W. Shafer. 1994. The prevalence of anti-IgE antibodies in 1000 volunteer blood donors. *J. Allergy Clinical Immunol.* 93:282.
- Ray, D.T., D.A. Dierig, A.E. Thompson, and T.A. Coffelt. 1999. Registration of six guayule germplasms with high yielding ability. *Crop Sci.* 39:300.
- Siler, D.J. and K. Cornish. 1994. Hypoallergenicity of guayule rubber particle proteins compared to *Hevea* latex proteins. *Indust. Crops Prod.* 2:307–313.
- Teetor, V.H., D.T. Ray, and M.E. Veatch. 2003. Concentration of resin, rubber, and guayulins in various parts of guayule plants. *AAIC Annu. Meeting Prog. Abstracts.* p. 28.
- Thompson, A.E. and D.T. Ray. 1988. Breeding guayule. *Plant Breeding Rev.* 6:93–165.
- Veatch, M.E., D.T. Ray, and K. Cornish. 2003. Growth, rubber, and resin evaluation of two-year-old transgenic guayule. *AAIC Annu. Meeting Prog. Abstracts.* p. 29.
- Wagner, J.P. and W.W. Schloman, Jr. 1991. Processing. p. 261–286. In: J.W. Whitworth and E.E. Whitehead (eds.), *Guayule natural rubber*. Office of Arid Lands, Univ. Arizona, Tucson.