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Final Quarterly Progress Report
(April - June 1992)

Biomass Energy Systems and Technology Project

Winrock International Institute for Agricultural Development

by

Hawaiian Sugar Planters' Association

March 1993

Cooperative Agreement No. 572-91-6

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Appendix A. Independent Laboratory Results for Egyptian Cane Residue Samples

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CANE ENERGY CONFERENCE
HSPA Project No. 5349

The International Conference on Energy from Sugarcane held September 10-13, 1991, in Hilo, Hawaii, was held during the first quarter of the funding period. HSPA staff contributing papers were Robert Osgood, Stephanie Whalen, Lance Santo, and Lee Jakeway. Draft papers were presented in the July-September 1991 Quarterly Progress Report.

At this writing, a cane energy network is being considered for establishment whicn is an outgrowth of the conference held in Hawaii. An inaugural meeting of a steering committee for the network is being planned for April 26-28, 1993, in Mauritius.

CANE RESIDUE PROCESSING
HSPA Project No. 5351

After discussion with the Winrock International Project Director regarding budget constraints, it was mutually decided to end activity for this project. Therefore, there are no results to report for this quarter. Please refer to previous quarterly reports for activity conducted on this project.

NEW/ONGOING CANE RESIDUE RECOVERY (CRR) ACTIVITY
HSPA Project No. 5352

Analyses for the cane residue samples obtained in Egypt in early 1992 were completed. The analyses performed were for moisture, ash, ash mineral analysis, N, P, K, calorific value, and ash fusion temperature. The complete independent laboratory results are given in Appendix A. Moisture results were determined by HSPA. Sample 3 was actually a composite of samples 1 and 2. The results for ash, calorific value, and tissue nutrient analysis are in ranges that were reported in the first quarterly progress report (Appendix I, International Cane Energy Conference Papers). Extrapolating from the results in Table 1 and using a recoverable cane residue yield in Egypt, estimated to be 8 t/ha, the equivalent amount of nutrients removed would be 40.8 kg N, 3.4 kg P, and 56.0 kg K, which is comparable to values obtained by Santo (First Quarterly Report, Appendix I, Agronomic Issues in Sugarcane Residue Collection). The initial deformation ash fusion temperature was somewhat lower compared to the published value of 2012°F (Jakeway, 1992 "Use of Baled Cane Residue for Off-Season Fuel," ISSCT Proceedings) but fusion temperatures at higher stages of deformation were somewhat higher.

Ash mineral analysis was performed for sample 3 only with the complete results given in Appendix A. The major mineral constituent of ash according to the results is silicon at 66.97%. Sodium was also high at 12.47%. Results for phosphorus as P_2O_5 and potassium as K_2O were quite different compared to the tissue analysis results. These results suggest that for a dry-matter yield of 8 t/ha with 12.9% ash content, the amount of nutrients removed from the field is equivalent to approximately 63.1 kg/ha of P and 1.3 kg of K if ash is not returned to the field. If concentrated dumping of ash were to occur then there may be ground contamination by trace metals such as aluminum, barium, magnesium, manganese, and titanium. The most likely scenario for disposing of the ash would be to return it to the field from where it originated.

The ongoing CRR activity planned for Jamaica in the early part of 1992 was postponed due to lack of funding.

Table 1. Summary of results for Egyptian cane residue samples.

	Sample No. 1	Sample No. 2	Sample No. 3	Average
Analysis				
Moisture (% wet basis)	7.76	6.77	--	7.27
Ash (% dry basis)	--	--	11.98	--
Calorific value (Btu/lb)	--	--	6755	--
Tissue Nutrient Analysis				
Nitrogen (ppm)	2100	7400	--	4750
Phosphorus (ppm)	67	730	--	399
Potassium (ppm)	4500	8500	--	6500

Ash Fusion Temperatures (°F) in Reducing Atmosphere for Sample 3

Initial Deformation Temperature (IT)	1777°
Fusion (Softening) Temperature (ST) H/W	2465°
Fusion (Softening) Temperature (HT) ½ H/W	2563°
Fluid Temperature (FT)	2607°

ROOT BIOMASS STUDIES ON SUGARCANE AND TREES HSPA Project No. 5353

These studies were initiated to develop root sampling methodologies and to estimate root biomass for sugarcane and trees. These root studies are being conducted in conjunction with a research project on sugarcane C-factor development for revision of the Universal Soil Loss Equation. Preliminary data on sugarcane root biomass at 6 and 12 months of age were reported in the July-September 1991 and January-March 1992 quarterly progress reports. Also, root distribution and sampling method development for the tree species *Leucaena leucocephala* was reported in the October-December 1991 quarterly progress report. In this final quarterly progress report, recommendations for sampling methodologies and root biomass estimation models for sugarcane and two species of trees are reported in detail in Appendix B.

The sampling methods developed for both sugarcane and trees involve digging a trench which spans the minimum representative soil profile for root sampling (i.e., from the drip line to the middle of the interrow for sugarcane and from the tree trunk to the middle of the interrow). The trenches should be dug to maximum rooting depth which is determined as the depth beyond which no roots are visible on the soil profile. Since a significant amount of biomass is associated with the below ground stem and large roots attached to it, these should be excavated, cleaned and weighed. The smaller roots in the rest of the profile are most accurately estimated by carefully removing and weighing blocks ("zones") of soil, removing roots larger than 1 mm in diameter, then thoroughly mixing and subsampling the soil for collection of small roots by sieving. The roots should be oven dried and a subsample ashed in a muffle furnace to determine dry weight and soil contamination, respectively. Augers can be used to core into the soil profile as a simpler alternative to zone sampling. Also, root biomass estimation models were developed for estimating total root biomass from more easily measured data such as root biomass in the stump zone and root number counts on the soil profile.

Below ground biomass for sugarcane was estimated to be about 6 T/ha at 6 months and about 11 T/ha at 12 months, while below ground biomass for *Leucaena leucocephala* and *Eucalyptus camaldulensis* were both estimated to be about 60 T/ha for five year old trees. Therefore, the trees seemed to have significantly more below ground biomass due to greater root growth and/or slower decomposition and turnover of the tree root systems. However, sampling sizes were very limited, especially for the trees, so the numbers reported should be considered to be only rough estimates. Also, these root biomass estimates are specific to the crop growth conditions for this study. The sugarcane was grown under drip irrigation on soil with a compacted subsoil (at >30 cm depth) which produced a shallow root system. The trees, which were grown at a different site from the sugarcane, were drip-irrigated during establishment but irrigated infrequently during the five years of growth; this produced very deep rooting on the well-drained soil. Rooting patterns and total amounts of roots are clearly dependent on site and crop management conditions, as well as species. Therefore, it is recommended that the root sampling and biomass estimation methods presented be considered for use in other sites which are representative of rainfed or furrow-irrigated and unmechanized conditions.

IMPACT OF MULTIPLE HARVEST RESIDUE REMOVAL ON SOIL FERTILITY AND SUGARCANE YIELD HSPA Project No. 5354

The agronomic study could not be done during the funding period because of travel restrictions to Thailand. However, this activity was eventually funded by Winrock International through a grant from the Rockefeller Foundation in the latter part of 1992. Mr. Lance Santo submitted a report to Winrock International under a separate arrangement.

FINAL BUDGET STATUS

The following reflects the final budget status as of October 8, 1992.

Project Title	Budget	Expenses		Balance (over)
		Quarterly	Accumulated	
Cane Energy Conference	$26,500	0	$28,105	($1,605)
Cane Residue Processing	11,415	0	2,962	8,453
New/Ongoing CRR Activity	51,380	$5,464	45,717	5,663
Root Biomass Studies on Sugarcane and Trees	49,410	21,331	40,420	8,990
Agronomic Impact Study	11,318	0	0	11,318
TOTAL	$150,023	$26,795	$117,204	$32,819

APPENDIX A

Independent Laboratory Results for Egyptian
Cane Residue Samples

HUFFMAN
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Quality Analytical Services Since 1936
4630 Indiana Street • Golden, CO 80403
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CUSTOMER #:
03662

DATE 6/24/92
LAB# 189492
P.O. 92-1203
RECD 06/04/92

ANALYSIS REPORT

LEE A. JAKEWAY
HAWAIIAN SUGAR PLANTERS
P. O. BOX 1057
AIEA HI 96701

SEQUENCE/ SAMPLE ID	01 1	02 2	03 3
NITROGEN------%	0.21	0.74	
PHOSPHORUS--PPM	67.	730.	
ASH----------%			11.98
BTU/LB			6755.
POTASSIUM---PPM	4500.	8500.	

ASH FUSION TEMPERATURES (DEG F) REDUCING ATMOSPHERE FOR SAMPLE 3:

IT	ST	HT	FT
1777	2465	2563	2607

THE BTU/LB RESULT IS THE GROSS HEATING VALUE.

ALL ABOVE RESULTS ARE ON AN AS RECEIVED SAMPLE BASIS.

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DATE: 06/24/92

LEE A. JAKEWAY, P.E.
HAWAIIAN SUGAR PLANTERS' ASSOCIATION
P. O. BOX 1057
AIEA, HI. 96701

ASH ANALYSIS

SEQUENCE/ 03/
SAMPLE #: 3

MINERAL ANALYSIS	% WT ASH BASIS
Aluminum, % as Al_2O_3	0.33
Barium, % as BaO	0.018
Calcium, % as CaO	7.06
Iron, % as Fe_2O_3	2.83
Magnesium, % as MgO	2.96
Manganese, % as MnO_2	0.20
Phosphorus, % as P_2O_5	6.10
Potassium, % as K_2O	0.13
Silicon, % as SiO_2	66.97
Sodium, % as Na_2O	12.47
Strontium, % as SrO	0.04
Titanium, % as TiO_2	0.03

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APPENDIX B

Report on HSPA Project No. 5353

ROOT BIOMASS STUDIES ON SUGARCANE AND TREES
HSPA Project No. 5353

I. SUGARCANE ROOT SAMPLING AND BIOMASS ESTIMATION

Information on sugarcane root systems is limited due to the difficulties in such studies, especially the intensive labor requirements for sampling and processing root systems. Profile wall and monolith methods have been used to determine root biomass, root length, and other root measurements in various crops (Boehm, 1979; Caldwell and Virginia, 1989). However, there has not been an adequate evaluation of the accuracy and labor requirements of these procedures for sugarcane. In this study, sampling depth required for adequate root recovery at different growth ages, errors in subsampling soil in the sampling zone, root separation from soil by different approaches, and correction of soil contamination in the root sample were evaluated in an attempt to establish the necessary procedures for sugarcane root sampling and root biomass estimation.

Also, it is desirable to develop simple and reliable methods to estimate root biomass from more easily measured parameters. Auger sampling and estimation of root biomass from surface soil is a common method (Boehm, 1979; Caldwell and Virginia, 1989) and requires relatively little labor. The hand auger is a readily available tool which is simple to use for sampling surface roots but is difficult to use for sampling roots deep in the soil. Cores taken to two meters depth would normally be needed for deep rooting crops such as sugarcane (Ball-Coelho et al., 1992).

Root number counting and mapping on the surface of soil profile has frequently been used to describe root distribution in the soil (Boehm, 1979). However, little attention has been paid to the possibility of using root number counting to estimate root biomass. This would be a very simple method for root biomass estimation if the accuracy were acceptable. Sampling only the top portion of soil profile may be another way to estimate root biomass in the whole profile. Likewise, there is no record of an attempt to develop such a method in the literature.

In this study, different methods for estimation of sugarcane root biomass from relatively simple measurements were developed and evaluated.

Materials and Methods

A sugarcane growth experiment conducted at the Hawaiian Sugar Planters' Association, Kunia Substation, on the island of Oahu, was chosen for this study. The soil at the site is classified as a clayey, kaolinitic, isohyperthermic, Typic Torrox. Two commercial sugarcane varieties, H73-6110 and H78-7234 were planted using drip irrigation and in a paired cane row planting system. The row spacing was 91 cm between paired rows, which had one drip line between them, and 183 cm between double rows. The experimental design was a split plot with two sugarcane varieties as main plots and harvest periods (growth ages) as

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subplots. There were three replications. Weekly irrigation and monthly fertilization were carried out according to usual Hawaiian plantation practices for drip irrigated fields.

After the harvest of aboveground biomass, trenches were dug perpendicular to the sugarcane rows from the drip irrigation line to the middle of interrow and to the maximum rooting depth (Figure 1). The vertical profile wall was smoothed and roots on the surface of the profile wall were carefully exposed by removing about 5 mm of soil with a forked scraper. The profiles were divided into sampling zones using a grid, on which visible roots were counted. The root number data were subsequently used to determine root biomass sampling depth to adequately sample the whole root system. Root counting and sampling zone sizes as well as depths in the soil profiles at different growth ages are shown in Figure 1. After soil in the sampling zone was taken and weighed, roots larger than 1 mm in diameter were removed by hand. The soil was then thoroughly mixed and a 4.5 kg soil subsample was taken for the estimation of small root biomass by washing through a sieve. This subsampling procedure was evaluated by taking 5 subsamples in one zone at each depth down to 76 cm at cane ages of 8 and 12 months. During sampling, the underground stems in sampling zones 2 and 3 were also saved for washing and dry weight determination.

Subsamples were stored by freezing until the washing could be done. Freezing and thawing also helped to break up soil aggregates, which facilitated separation of roots. During thawing, water was added to soften soil samples overnight. Soil samples were then washed through a 0.14 mm^2 pore size sieve to separate out the roots. Using a 0.05 mm^2 pore size sieve in the washing was also tested on eight month old cane for one profile.

The roots were dried at 60° C to constant weight. A portion of the dried root was weighed and ashed in a 550°C muffle furnace for eight hours and the residue (which included soil particles still remaining in the washed root sample, soil still adhering to the surface of the washed root and root ash) was weighed for the correction of soil contamination. The root ash weights in the residue of one profile were also measured using 50 ml of 0.2 molar hydrochloric acid to treat each sample for ten minutes. Those root processing procedures mainly followed the procedures of Willard and McClure (1932).

Zone sampling was used as a standard method for comparison of root biomass estimated by different methods. At 12 months of cane age, one soil profile for each sugarcane variety was chosen to test the auger sampling method. Before taking zone samples in the soil profile, two hand auger cores (8 cm in diameter and 10 cm long) were taken perpendicular to the surface of profile wall for each sampled zone and were processed in the same way as zone samples for root biomass measurement. Data were analyzed by Table Curve 3.1 curve fitting software (Jandel Scientific, 1992) to establish regression models for prediction purposes. Equations were selected based mainly on increases in the correlation coefficients (r^2).

Results and Discussions

Sampling depth

Sampling depth is a critical consideration for root sampling to insure good estimates of the entire root system. For this purpose, the trenches were dug to maximum rooting depth and roots on the surface of the soil profiles were carefully exposed to clearly show the root distribution on the profile walls. Roots on the profile walls were then counted to that depth and the proper root sampling depth was decided by including more than 95% of root number into the sampling depth. At this depth, it was estimated that at least 95% of root biomass could be recovered at all growth ages (Table 1). Therefore, the sampling depths used for different growth stages were 61 cm at 2 to 4 months, 76 cm at 6 to 8 months and 91 cm at 10 to 12 months.

Root separation

Researchers usually separated roots from soil by washing soil samples through a sieve. This wet sieving is very time consuming so that some researchers have omitted this procedure by using dry sieving. Previous research in Hawaii, for example, used a screen with 36 mm^2 (6 mm or 1/4 inch) pore size to separate roots from soil (Lee, 1926). Wet sieving, however, is necessary to separate roots from soil as completely as possible. A large portion of roots (small roots), consisting of about 60% of total roots, still remained in soil after simulating dry sieving by removing the roots (large roots) by hand (Table 2). It is obvious that the dry sieving would result in significant loss of small roots.

Researchers have used sieves with different pore sizes for wet sieving, varying from 2 mm^2 to 0.03 mm^2 (Boehm, 1979). The smaller pore sizes increased the root recovery but also increased the washing time. In this study, about 90% of roots in the soil sample could be recovered by using a 0.14 mm^2 (0.417 mm or 0.0164 inch) sieve assuming 100% of root recovery was achieved by a 0.05 cm^2 (0.221 mm or 0.0087 inch) sieve. This recovery is accurate enough for most root studies. Using sieves with pore sizes greater than 0.14 mm^2 are not recommended due to loss of small roots. Although using a 0.05 mm^2 sieve significantly increased washing time, this pore size would be necessary for more accurate studies because 10% more roots are recovered and these fine roots play an important role in nutrient absorption.

Subsampling error

It is impractical to wash all the soil in a sampling zone due to the high labor requirement for this large amount of soil (about 40 kg of soil in this study). Therefore, it is necessary to subsample the soil from the sampling zone. However, the variability of this subsampling procedure must also be considered. The sampling variance was obtained by analysis of variance and used to calculate the subsampling error using the equation (Freese, 1967):

$$E^2 = t^2 S^2 / n$$

Where: E = sampling error
t = the student's t value at a 95% of confidence level for n-1 degrees of freedom
S^2 = Mean Square error

In this study, the variability of root biomass in subsamples was significant and it was found that removing roots larger than 1 mm in diameter, greatly reduced this variability. The standard deviation and coefficient of variation of root biomass in subsamples in the sampling zone (Table 3) as well as the error mean square and coefficient of variation of all subsamples in the 6 selected sampling zones (Table 4) were all reduced by removal of large roots. When large roots were removed, the margin of error at 95% confidence was reduced from 24.2% to 15.3% for one subsample (Figure 2). Taking one subsample after removing large roots is recommended for most root studies, with a 15.3% margin of error, because taking two subsamples doubled root washing and processing work, but reduced subsampling margin of error only to 10.8%. If greater accuracy is critical, however, two subsamples should be taken. It is not necessary to take more than two subsamples because further reduction in margin of error is slight.

Soil contamination correction

Even after thorough washing, soil can still remain on the root samples and cause error. However researchers do not always correct for this soil contamination since the procedure is time consuming. In this study, soil contamination was so high, due to the heavy clay soil and the small pore size sieve used, that it could not be over-looked and was corrected by ashing root samples in a muffle furnace. The average soil contamination was 12% for large roots, 39% for small roots and 32% for total roots (Table 5), which was higher than the results obtained by Willard and McClure for bluegrass roots (1932). The soil contamination consisted of soil particles in the root sample and soil adhering to the root surface.

Removing soil particles from the root sample before weighing or washing root sample intensively was tested and those procedures could reduce soil contamination by only about 8%. Therefore, there was still a significant amount of soil adhering to the root surface and it could only be corrected by ashing. However, it was unnecessary to determine root ash weight after ashing by treating ash with hydrochloric acid (Willard and McClure, 1932) since ash weight is only 3.2% of the net root weight. This time the consuming step was omitted to simplify the procedures.

Accuracy of root biomass estimates

If the final root biomass, as estimated by the zone sampling procedures, is considered to be 100%, the various sources of potential error come from root ash in the muffle furnace residue (+3.2%), nonrecovery of fine roots in sieving (+10.8%), margin of error in subsampling roots in the sampling zone (+/-15.3%) and roots below the sampled zones (+1.5%). Taking all these factors into

account, the accuracy of root biomass estimates range from about 1.7% overestimation to 33.8% underestimation. Given the difficulties in sampling and processing, this seems to be acceptable accuracy. If root ash were acid treated, a 0.05 mm^2 sieve was used in washing and two subsamples were taken in the sampling zone, the accuracy of root biomass estimates would increase and could range from 9.5% overestimation to 12.4% underestimation.

Auger sampling

Auger sampling resulted in lower root biomass estimates than zone sampling for varieties H73-6110 and H78-7234 by 96% and 79%, respectively (Table 6). The low estimation of root biomass by auger sampling all of the zones (method A) resulted from low estimates of root biomass in the stump zones (30 cm * 91 cm * 30 cm in length * width * depth) under sugarcane stalks in the soil profile. The non-stump zones were less variable and auger sampling can be used more accurately to estimate root biomass (Figure 3). Therefore, a combination of zone sampled root biomass in the stump zones and auger sampled root biomass in the non-stump zones (method D) gives highly accurate estimates of root biomass with 102% and 101% of zone sampling estimates for varieties H73-6110 and H78-7234, respectively (Table 6).

Stump zone prediction

Root biomass in the stump zones of the soil profile can be sampled and processed in a reasonable amount of time using zone sampling. The relationships between root biomass in different sized stump zones and the total root biomass were very strong (Table 7). Most of roots, especially the large roots with diameters greater than 1 mm, concentrate under stumps. These large roots are difficult to sample accurately using other methods, such as auger sampling or root number counting. Stump zones 30 cm * 91 cm * 30 cm in length * width * depth were selected to predict total root biomass owing to a high correlation coefficient and reasonable labor requirements. This method (method B), resulted in estimates of root biomass for varieties H73-6110 and H78-7234 which were 105% and 94% of the zone sampling estimates, respectively (Table 6).

Root number prediction

Root number counting on the surface of profile wall is the easiest method to estimate root biomass and provides a clear picture of root distribution in the soil. Root biomass prediction equations from different cane ages for both varieties all had significant correlation coefficients. There were no significant differences between varieties or cane ages in slopes (b) or Y-intercepts (a) of the equations for 4 to 12 month old sugarcane (Table 8). Therefore, it is appropriate to use the equation for both varieties at 4-12 month old cane ages (method C) as the root biomass prediction model. However, estimates of root biomass by method C for variety H73-6110 and H78-7234 for 12 month old cane were 79% and 133% of zone sampling estimates, respectively (Table 6). This method was not accurate because of failure to predict root biomass in the stump zones accurately. Therefore, a combination of zone sampled root biomass in the stump zones and

root biomass prediction from root number in the non-stump zones (method F) gave higher accuracy.

Root biomass estimates

Root biomass estimates using zone sampling procedures and estimates of underground stem biomass for 6 and 12 month old sugarcane are presented in Table 9. At both ages, the majority of the root systems were present in the top 46 cm of the profile. This relatively shallow rooting is probably related to the use of a drip system for irrigation and fertilization and the resulting low soil moisture and nutrient stresses on the crop. It is expected that unirrigated sugarcane on deep soils would have both larger root systems and a greater proportion of total biomass in the root systems. These studies have continued for 18 and 24 month old sugarcane, which will be reported at a later time.

Conclusions

The procedures used in root sampling and processing greatly affect the measurement accuracy and the labor and time required. It is essential to evaluate and establish recommended procedures for a given crop to give acceptable accuracy and reasonable labor requirements. In this study, several interesting findings may be useful for root studies in other crops:

(1) Root number counted on the profile wall is a simple and reliable approach to determine proper root sampling depth with more than 95% of root number included in the sampling depth.

(2) It is necessary to separate roots from soil by washing soil through a 0.14 or 0.05 mm^2 pore size sieve.

(3) Removing large roots before taking a soil subsample can reduce subsampling error significantly.

(4) It is necessary to correct soil contamination in the root sample by ashing.

Besides the procedures evaluated in this study, soil profile replications in the sampling plot or field should be evaluated according to the variability of sugarcane growth along the cane row.

It is necessary to directly sample stump zones (30 cm * 91 cm * 30 cm in length * width * depth) under sugarcane stalks in the soil profile because most of the roots, mainly large roots with a diameter greater than 1 mm, concentrate in the stump zones. These large roots are difficult to estimate accurately by simple methods such as root number counting (method C) or auger sampling (method A). After sampling stump zones, however, root biomass can be estimated by direct prediction from stump zone root biomass (method B). Greater accuracy is obtained by combining directly sampled stump zone root biomass with root biomass in the non-stump zones estimated by either auger sampling (method D),

stump zone prediction (method E) or root number prediction (method F). The combination of stump zone sampling and auger sampling (method D) was the most accurate method and can be used as an alternative method to zone sampling in root studies under field conditions. Total root biomass estimation from stump zone root biomass (method B) is simple and can be used for survey purposes while root number counting (method F) is useful to characterize root distribution and for rough estimates of root biomass.

II. TREE ROOT SAMPLING AND BIOMASS ESTIMATION

A study of tree roots was done at the USDA Soil Conservation Service Plant Materials Center on the island of Molokai in June 1992. A preliminary sampling at the site in December 1991 was reported in the October-December 1991 Quarterly Progress Report. The site of the study is a biomass-to-energy research project conducted by the Hawaiian Sugar Planters' Association and funded by the State of Hawaii, Department of Business, Economic Development & Tourism (Osgood and Dudley, 1986; Dudley, 1990). The purpose of this tree root biomass evaluation is to provide estimates of tree root biomass for comparison to the sugarcane root biomass estimates. Due to time and budget limitations (mainly related to the cost of travel between the islands of Oahu and Molokai and to the great depths of the tree root systems) this study was envisioned as a limited assessment of tree root biomass to provide some rough estimates and to begin to develop root study methodologies.

Materials and Methods

Stands of five year-old *Eucalyptus camaldulensis* and *Leucaena leucocephala* trees at 2 m x 2 m spacing were selected for the study. The trees had been irrigated and fertilized using drip irrigation. One tree of each of the species was felled and trunk and leaf components were weighed. A 3.5 meter deep trench was dug next to each tree stump using a backhoe. After observation of the root systems, which were present to the bottom of the profile, it was determined that excavation of a complete soil profile to maximum rooting depth of the trees was impractical since the trees were so deep rooted. However, a good assessment of root distribution was possible for what appeared to be the majority of the root systems.

The roots were counted in the soil profile in 25 cm x 25 cm sampling zones, with separate counts for small roots (less than 2 mm in diameter) and larger roots (2 mm to 10 mm in diameter). A root auger was then used to collect 16 soil samples in the *Eucalyptus* trench and 12 soil samples in the *Leucaena* trench at various depths to develop models for estimating tree root biomass. Three auger samples (8 cm x 10 cm) were taken in each selected sampling zone. The auger samples were washed and processed similarly to the procedure described for sugarcane samples in the previous section. The tree stumps and buttress roots were also excavated and weighed.

Table 1. Root number and root biomass as percentage of total root at the sampled depths and the cane ages.

Variety	Age (month)	Replications Measured		Percentage of Total Root						Measured depth (cm)	
				0-46 cm depth		46 cm—SD[a]		Below SD[a]			
		Number	Biomass	Number	Biomass	Number	Biomass	Number	Biomass	Number	Biomass
H73-6110	12	6	3	85.30	88.99	12.94	9.17	1.76	1.84	121	91
	8	1	1	90.47	94.89	8.70	4.17	0.83	0.94	91	76
	6	6	3	91.70	91.20	6.96	7.29	1.34	1.51	91	76
	4	2	1	96.74	96.43	2.58	1.10	1.35	1.47	91	61
	4-12			91.05	93.13	7.80	5.43	1.32	1.44		
H78-7234	12	5	3	86.22	85.10	12.91	13.94	0.87	0.96	121	91
	8	1	1	95.18	93.45	4.18	5.81	0.64	0.74	91	76
	6	6	3	88.03	91.56	9.32	5.54	2.64	2.90	91	76
	4	2	1	94.90	94.49	4.11	3.71	1.99	1.80	91	61
	4-12			91.08	91.15	7.63	7.25	1.53	1.60		

[a] SD = sampled depth (i.e., depth to which soil was actually sampled at each age).
[b] Root biomass below sampled depth were predicted by Y=0.003775+6.657X, where, Y=root biomass (mg/cm3), X=root number (roots/cm2), n=300, r^2=0.6341, from both varieties at 4 to 12 month growth ages.

Table 2. Root biomass separated from soil by different methods.

Age	Sampled Size	Replications	Separation methods			
			Hand[a]		Washing[b]	
(months)	(cm)		(g)	(%)	(g)	(%)
2	Topsoil (30*46*30)[c]	6	6.50	35.35	11.89	64.65
	Whole profile (30*91*46)[c]	1	19.02	40.07	28.45	59.93
12	Topsoil (30*91*46)[c]	6	75.76	40.03	113.47	59.97
	Whole profile (30*107*91)[c]	6	90.97	36.30	159.65	63.70

[a] Hand separation method involves removing roots larger than about 1 mm in diameter by hand.
[b] Washing separation method involves washing small roots through a 0.14 mm^2 pore size sieve after removing large roots.
[c] Length * width * depth in sampled size.

Table 3. Comparison of subsample variances for sugarcane root biomass in the sampled zones with large roots either remaining or removed.

Large roots	Zone No.	Depth	Mean	Standard Deviation	Coefficient of Variation
		(cm)	(kg/ha)	(kg/ha)	(%)
Remaining	3	0-10	697.2	56.46	8.10
	5	10-20	578.7	50.96	8.81
	7	20-30	181.2	16.19	8.93
	12	30-46	108.1	20.64	19.10
	14	46-61	111.5	8.35	7.49
	16	61-76	14.3	4.43	31.04
Removed	3	0-10	649.4	21.28	3.28
	5	10-20	531.1	33.84	6.37
	7	20-30	180.5	14.4	48.00
	12	30-46	462.8	38.68	8.36
	14	46-61	199.7	23.19	11.61
	16	61-76	22.6	1.32	5.83

Table 4. Analysis of variance for subsampling of sugarcane root biomass in the sampled zone.

Source of Variation	df	Mean Square: Large roots remaining	Mean Square: Large roots removed
Sampled zone	5	401598	293151
Error	24	1094	640
Coefficient of Variation (%)		11.7	7.4

Table 5. Soil contamination of field sampled sugarcane roots. (Data presented are averages of six plots for each age.)

Age	Root Size	Contaminated Root Weight	Soil & Ash Weight After Burning	Net Root Weight	Contamination
(month)		----------(g/profile----------			(%)
2	Large	7.16	0.67	6.49	9.36
	Small	22.40	10.51	11.89	46.91
	Total	29.56	11.18	18.38	37.81
12	Large	107.17	16.19	90.97	15.11
	Small	230.45	70.73	159.72	30.69
	Total	337.62	86.92	250.69	25.75
Average	Large	57.16	8.43	48.73	12.23
	Small	126.42	40.62	85.81	38.80
	Total	183.59	49.05	134.54	31.78

[a] Large roots were first removed by hand and small roots are the roots remaining after washing with a 0.14 mm^2 sieve.

Table 6. Comparison of root biomass estimated by different methods to the standard method of zone sampling for two varieties of 12 month old sugarcane.

	H73-6110		H78-7234	
Method	Root Biomass (kg/ha)Zone	Percent of Method	Root Biomass (kg/ha)	Percent of Zone Method
Zone	5648	100.0	5018	100.0
A	5435	96.2	3983	79.4
B	5934	105.1	4733	94.3
C	4474	79.2	6685	133.2
D	5749	101.8	5060	100.9
E	5904	104.5	4681	93.3
F	5809	102.9	6286	125.3

Zone Direct estimation based on zone sampled root biomass.

A Direct estimation based on auger sampled root biomass.

B Direct estimation based on zone sampled root biomass in the stump zones. (kg roots/ha = 158.8 Sqrt(kg roots/ha) - 3847, n=17, r^2=0.9089)

C Direct estimation based on root count on the surface of the sampled zones. (mg roots/cm^3 = 0.003775 + 6.657 roots/cm^2, n=300, r^2=0.6341)

D Combination of zone sampled root biomass in the stump zones and auger sampled root biomass in the remaining zones.

E Combination of zone sampled root biomass in the stump zones and estimation of root biomass in the remaining zones based on root biomass in the stump zones. (kg roots/ha = 1331 Ln(kg roots/ha) - 8855, n=17, r^2=0.5107)

F Combination of zone sampled root biomass in the stump zones and estimation of root biomass in the remaining zones based on root number on the zone surface. (mg roots/cm^3 = 0.09803 + 3.638 roots/cm^2, n=204, r^2=0.5491)

Table 7. Relationship of root biomass in the stump zone to the total root biomass or to root biomass in the non-stump zone for sugarcane at 4-12 months.

Stump zone size (cm)	Equation[a]	r^2	Percent of Total Root Biomass	
Top to total:				
30*46*30	Y = (27.408 LnX-136.93)^2	0.8959	8	42.04
30*91*20	Y = (29.479 LnX-155.86)^2	0.8766	17	47.24
30*91*30	Y = 158.79 Sqrt(X)-3846.6	0.9089	17	64.09
30*91*46	Y = 149.19 Sqrt(X)-4062.6	0.9825	17	76.63
Top to remaining:				
30*46*30	Y = 1746.0 LnX-102000.	0.6782	8	
30*91*20	Y = (67.628-30856/X)^2	0.6279	17	
30*91*30	Y = 1330.5 LnX-8855	0.5107	17	
30*91*46	Y = 1867.6-2205712/X	0.7550	17	

[a] Y = total root biomass or root biomass in the non-stump zones; X = root biomass in the stump zones in the soil profile.

Table 8. Relationships of root number on the surface of sampled zones to root biomass in the sampled zones for different varieties and cane ages of sugarcane.

Age (month)	Variety	Equation[a]	n	r^2	95% Confidence Intervals Slope	95% Confidence Intervals Y-intercept
4	H73-6110	Y=6.633X+0.1473	15	0.6645	3.805 to 9.461	-0.1982 to 0.4928
	H78-7234	Y=9.363X-0.0097	15	0.6752	5.468 to 13.259	-0.4429 to 0.4235
	Both	Y=7.778X+0.0899	30	0.6453	5.546 to 10.01	-0.1759 to 0.3457
6	H73-6110	Y=5.750X+0.0797	54	0.7604	4.852 to 6.649	-0.0322 to 0.1916
	H78-7234	Y=5.357X+0.1220	54	0.5201	3.925 to 6.789	-0.0483 to 0.2924
	Both	Y=5.561X+0.1006	108	0.6302	4.740 to 6.381	0.0007 to 0.2006
8	H73-6110	Y=8.807X-0.3816	18	0.8045	6.502 to 11.113	-0.8806 to 0.1174
	H78-7234	Y=6.415X+0.0457	18	0.4164	2.382 to 10.448	-0.7151 to 0.8066
	Both	Y=7.521X-0.1294	36	0.5878	5.326 to 9.716	-0.5750 to 0.3162
12	H73-6110	Y=7.877X-0.1234	63	0.7037	6.569 to 9.186	-0.326 to 0.0791
	H78-7234	Y=5.591X+0.0321	63	0.6844	4.619 to 6.563	-0.1156 to 0.1798
	Both	Y=6.765X-0.0483	126	0.6748	5.931 to 7.600	-0.1763 to 0.0798
4-8	H73-6110	Y=7.006X-0.0203	87	0.7594	6.156 to 7.857	-0.1461 to 0.1056
	H78-7234	Y=6.172X+0.0943	87	0.4726	4.766 to 7.578	-0.0959 to 0.2847
	Both	Y=6.602X+0.0382	174	0.6057	5.800 to 7.404	-0.0754 to 0.1518
8-12	H73-6110	Y=8.065X-0.1688	81	0.7389	6.991 to 9.138	-0.3518 to 0.0142
	H78-7234	Y=5.909X+0.0216	81	0.5444	4.698 to 7.119	-0.1731 to 0.2163
	Both	Y=7.031X-0.0735	162	0.6443	6.215 to 7.846	-0.2087 to 0.0616
4-2	H73-6110	Y=7.395X-0.0653	150	0.7278	6.660 to 8.130	-0.1762 to 0.0455
	H78-7234	Y=5.848X+0.0746	150	0.5338	4.960 to 6.736	-0.0519 to 0.2012
	Both	Y=6.657X+0.0038	300	0.6341	6.081 to 7.234	-0.0808 to 0.0884

[a] Y = root biomass (mg/cm^3) in the sampled zones and X = root number ($roots/cm^2$) on the surface of sampled zones in the soil profile.

Table 9. Total above and below ground biomass of two sugarcane varieties at 6 and 12 months of age.

Soil Depth	6 months				12 months			
	H73-6110		H78-7234		H73-6110		H78-7234	
(cm)	kg/ha (%[a])							
0-10	1155	(26.6)	1043	(25.3)	1404	(21.7)	1174	(21.3)
10-20	991	(22.8)	1069	(25.9)	1644	(25.4)	1348	(24.4)
20-30	926	(21.3)	994	(24.1)	1457	(22.5)	1156	(21.0)
30-46	883	(20.4)	651	(15.8)	1268	(19.6)	1031	(18.7)
46-61	225	(5.2)	256	(6.2)	322	(5.0)	430	(7.8)
61-76	159	(3.7)	115	(2.8)	194	(3.0)	225	(4.1)
76-91					184	(2.8)	152	(2.8)
Total Root Biomass	4338		4127		6474		5517	
Above Ground Biomass	31222		28376		89801		78174	
Below Ground Stems	1804		1588		5081		5344	
Total Biomass	37364		34091		101356		90035	
Root/Total Biomass	0.116		0.121		0.064		0.061	

[a]Percent of root biomass in the total soil profile.

Table 10. Tree root biomass estimation models.

a. ***Eucalyptus camaldulensis***

Root Diameter Class	Equation[a]	n	r^2 Value
Small(< 2mm)	$Y = 0.0928\ e^{22.9\ x}$	16	0.781
Large (> 2mm)	$Y = 0.0834\ e^{118.7\ x}$	16	0.828
Small & Large	$Y = 0.151\ e^{20.9\ x}$	16	0.787

b. *Leucaena leucocephala*

Root Diameter Class	Equation[a]	n	r^2 Value
Small (< 2mm)	$Y = -0.0628 + 13.6\ x$	12	0.924
Large (2mm - 10mm)	$Y = -0.0586 + 127.1x$	12	0.721
Small & Large	$Y = -0.557 + 32.8\ x$	12	0.853

[a] Units: Y = mg roots/cm^3, x = number of roots/cm^2.

Table 11. Estimates of tree root biomass from root number counts on the soil profile wall. Total root biomass was calculated from separate regressions of small roots (< 2mm) and large roots (2mm - 10mm).

a. *Eucalyptus camaldulensis*

Depth	Distance From Tree Trunk (cm) 100-75	75-50	50-25	25-0	Sum	Percent
	(kg/ha)					
0-25	4093	11378	9351	5817	30639	62.85
25-50	2647	2131	3017	1931	9726	19.95
50-75	475	403	520	462	1859	3.81
75-100	234	250	175	319	978	2.01
100-125	170	154	146	160	630	1.29
125-150	136	136	149	157	577	1.18
150-175	130	136	141	136	543	1.11
175-200	126	146	148	137	557	1.14
200-225	160	170	152	130	612	1.26
225-250	134	141	131	154	560	1.15
250-275	119	130	126	133	508	1.04
275-300	132	121	127	145	525	1.08
300-325	156	135	127	138	556	1.14
325-350	119	117	125	117	478	0.98
Sum	8830	15548	14434	9936	48748	100.00

b. *Leucaena leucocephala*

Depth	Distance From Tree Tunk (cm) 100-75	75-50	50-25	25-0	Sum	Percent
	(kg/ha)					
0-25	2271	4476	3838	4747	15332	42.52
25-50	1655	2227	2694	2498	9074	25.17
50-75	1105	1212	1271	1867	5456	15.13
75-100	415	424	367	311	1517	4.21
100-125	263	266	272	189	990	2.74
125-150	218	100	148	171	637	1.77
150-175	19	62	53	0	134	0.37
175-200	114	33	80	194	420	1.17
200-225	80	83	60	60	282	0.78
225-250	62	0	0	0	62	0.17
250-275	126	133	372	196	827	2.29
275-300	325	110	0	39	474	1.31
300-325	260	210	133	139	741	2.06
325-350	42	5	62	0	110	0.30
Sum	695	9341	9351	10410	36056	100.00

Table 12. Estimated total below ground biomass per hectare for two tree species. (The estimates for stumps assumes a complete stand of 2500 trees per hectare).

Tree Species	Roots	Stumps	Total
	----------(kg/ha)----------		
Eucalyptus camaldulensis	48,748	15,100	63,848
Leucaena leucocephala	36,056	24,015	60,071

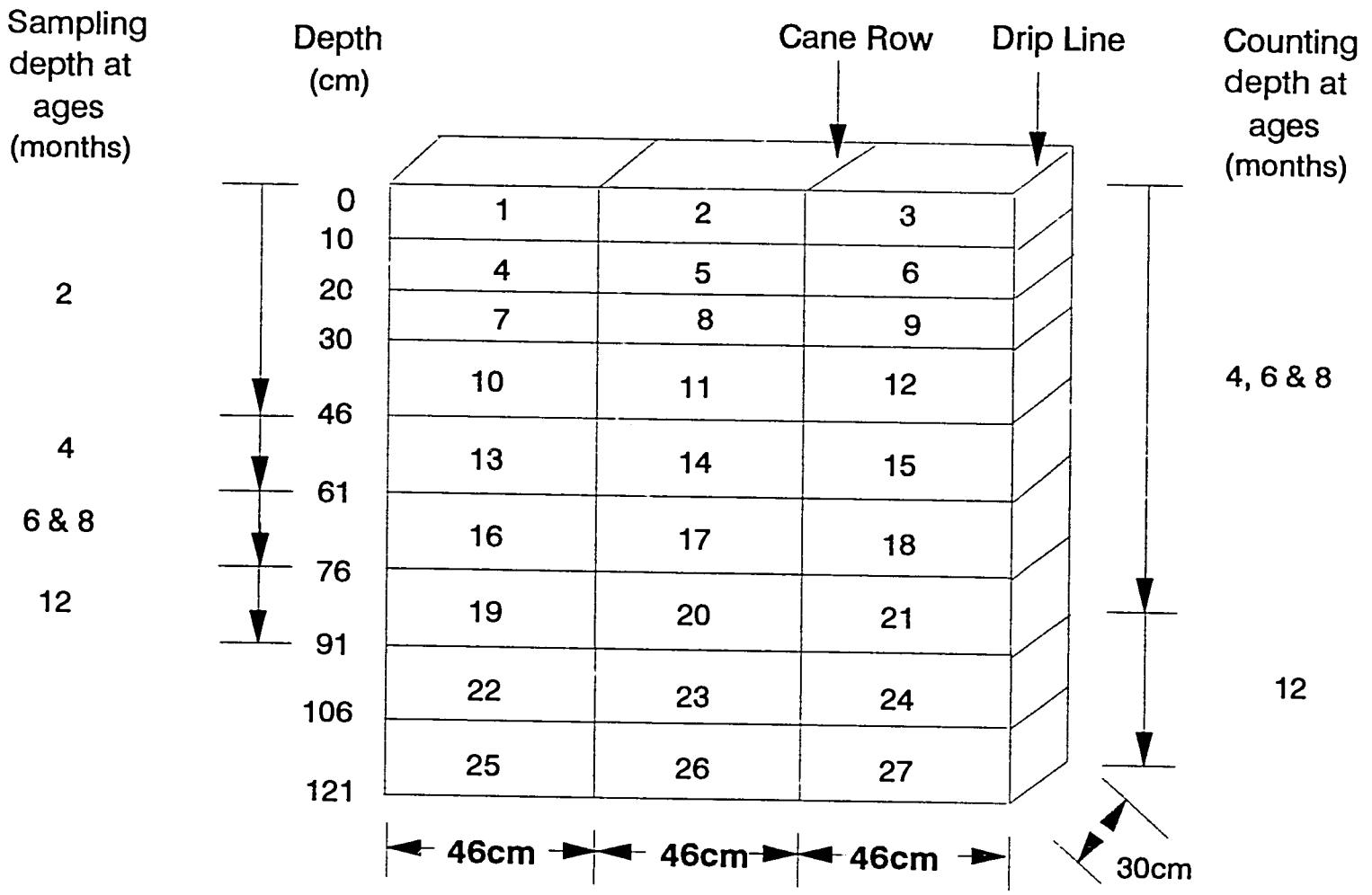

Figure 1. Diagram of zones for root biomass sampling and root number counting on the soil profile at different growth ages for sugarcane. Data in the zones are the zone numbers.

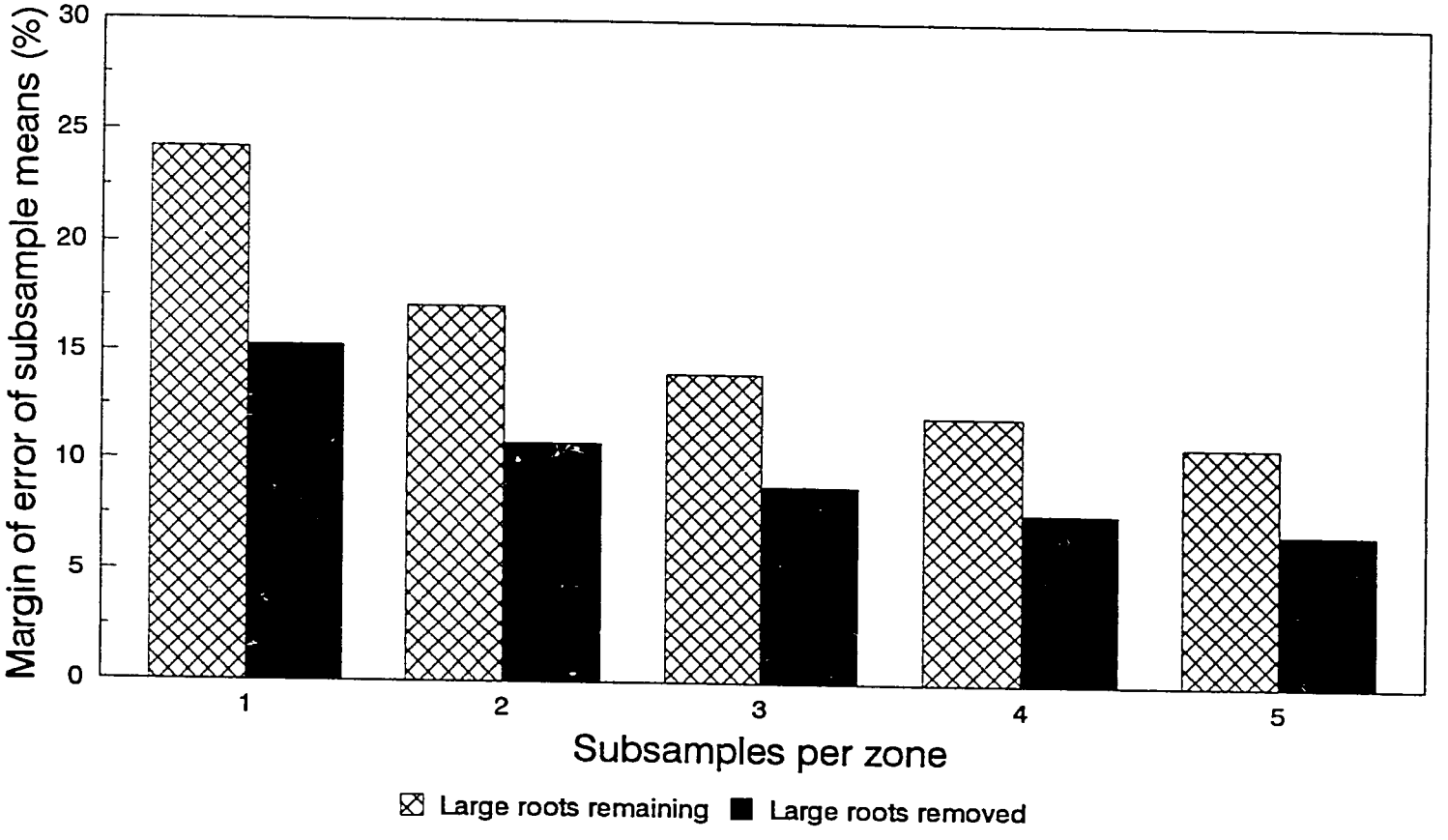

Figure 2. Comparison of the margin of error of the sample means at the 95% confidence level for different numbers of subsamples with large roots either remaining or removed from the zone sampled soil.

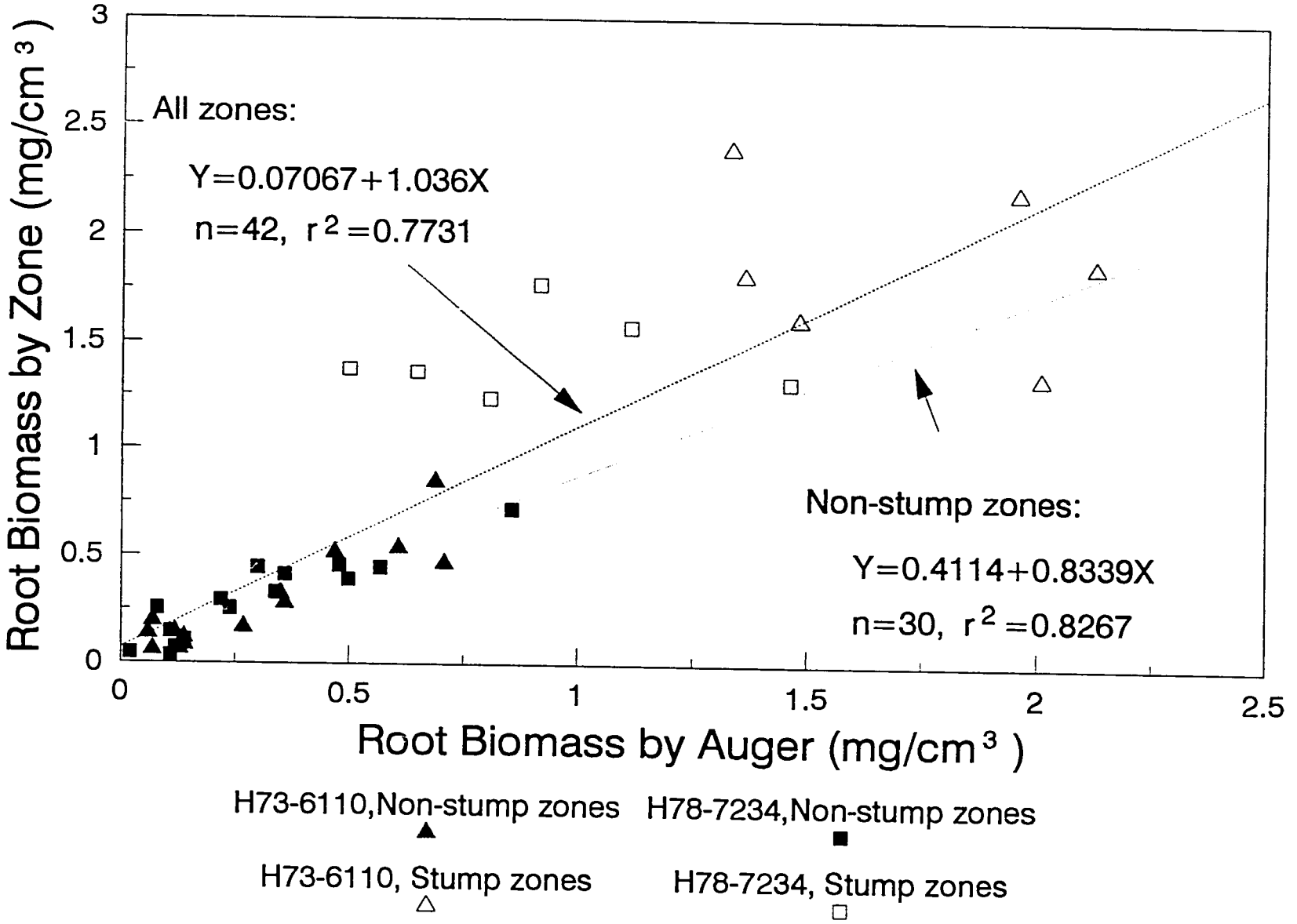

Figure 3. The relationship of root biomass sampled using augers (either including or not including stump zones) to root biomass estimated by zone sampling. Stump zones are 30cm x 91cm x 30cm in length x width x depth beneath stalks in the profile.

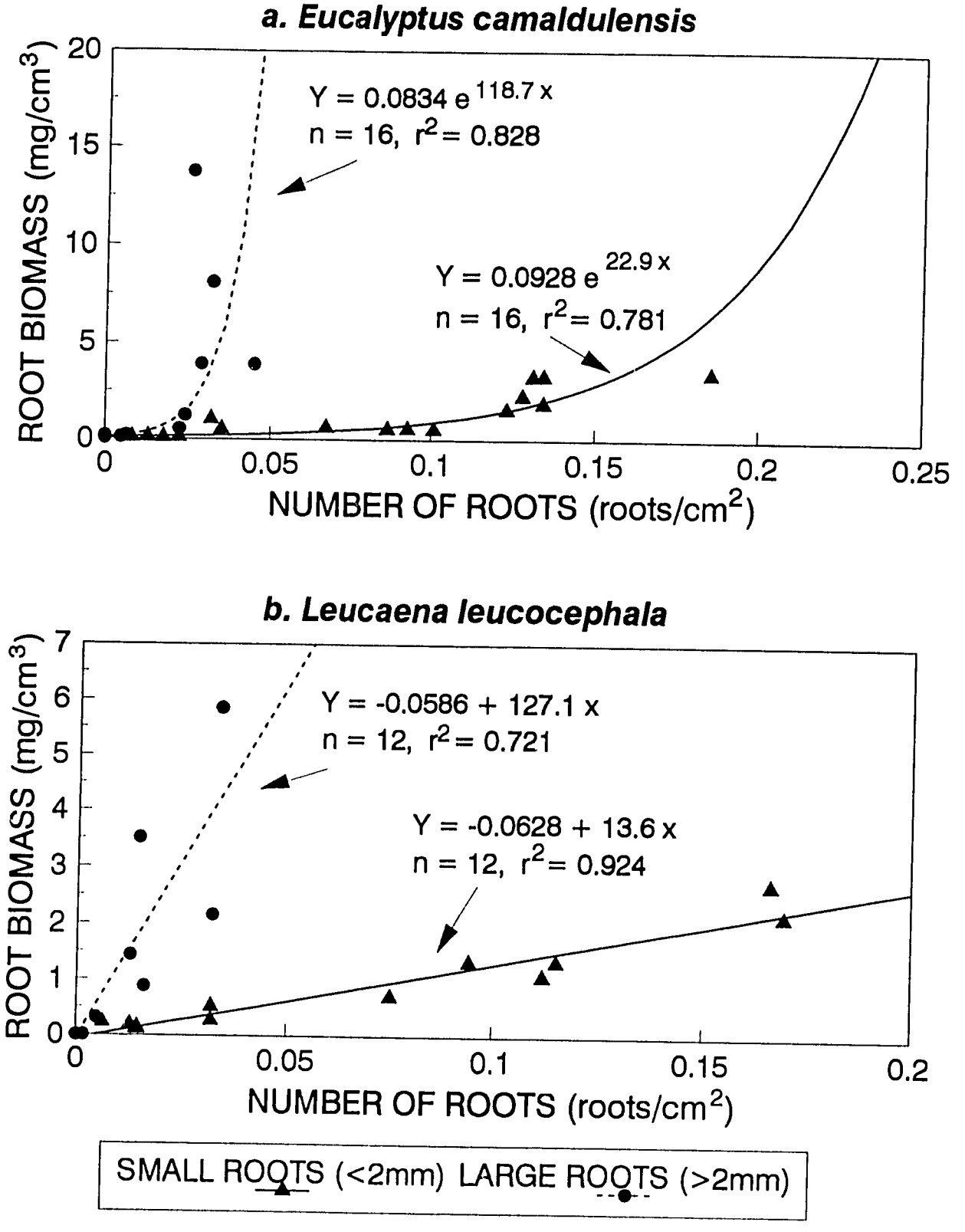

Figure 4. The relationship of root number on the surface of sampled zones to root biomass in the sampled zones for two tree species.