Toward Standardized Leaf Sampling for Foliar Nutrient Analysis in Breadfruit

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Summary. **Breadfruit (*Artocarpus altilis*) cultivation is gaining momentum throughout the tropics due to its high yield and nutritious fruit. One impediment to expanding production of breadfruit is the lack of agronomic research related to production management. We examined foliar nutrient concentrations of different leaf positions and leaf parts to assess within- and between-tree variance to inform an effective sampling protocol. We further validated the sampling protocol on 595 trees at 87 sites that were assessed for yield and productivity. Foliar nutrients differed significantly by categories of productivity. For the first time, breadfruit-specific standards of foliar nutrient concentrations are presented for consideration. In conclusion, we recommend that foliar sampling use petioles harvested from leaves in the third position from the branch tip using sun-exposed leaves in the midcanopy of each tree.**

Breadfruit has experienced an exponential increase of plantings in Hawai'i (Langston and Lincoln, 2018) and an increase in distribution and cultivation globally (Lincoln et al., 2018). The Food and Agriculture Organization of the United Nations (2009) has recognized breadfruit as one of 35 priority crops for its tremendous potential to improve global food security, human nutrition, and climate-smart agriculture in the tropics (Lucas and Ragone, 2012; McGregor et al., 2016). As one of the few staple foods that grow on long-lived perennial trees, breadfruit has potential to dramatically shift cultivation practices in tropical regions away from annual crops. Breadfruit is highly productive, with consistent yields of at least 5 $t \cdot ha^{-1}$ edible dry weight [see Lincoln et al. (2018) for compilation of reported yields] and was an important element of Hawaiian food systems in the past (Lincoln and Ladefoged, 2014; Winter et al., 2018). Despite the growing interest and awareness of the production potential of breadfruit, it remains underused and suffers from significant lack of research investment (Lincoln et al., 2018; Ragone, 2007). Thus, substantial gains in breadfruit yield are probable with relatively little agronomic research and breeding effort (Sraffa, 2005; Willcox, 1954).

Foliar nutrient analysis is a well-established method (Munson and Nelson, 1990) to assist in the diagnosis of nutrient-related problems (deficiencies, toxicities, imbalances, etc.) of both annual and perennial crops. Assessment of foliar nutrient concentrations can be applied to inform fertilizer management, rule out nutrition as a source of a production variability, and assess the impact of management techniques on the nutrient status of crops. Foliar nutrient analyses are employed through tissue-sampling methods, which are contingent on sampling location, plant part selected, and the stage of growth. Specific sampling protocols may be dependent on the crop type and purpose (Munson and Nelson, 1990). Furthermore, optimal nutrient levels must to be established for each individual crop. Although important work has been done for many fruit crops (Jones, 2001), including species related to breadfruit (Poovarodom et al., 2000; Sun et al., 2015; Tawinteung et al., 2001), no studies investigating foliar sampling protocols for breadfruit were identified (Lincoln et al., 2018).

Using data from commercial and research orchards in Hawai'i, we explored foliar sampling methods of breadfruit to inform a sampling protocol. Sampling entire breadfruit leaves is impractical due to their size (we have measured leaves up to 1.4 m in length). Thus, implementing a method that uses a portion of a leaf is necessary. Our primary objective was to assess leaf location and leaf part for suitability in foliar nutrient diagnosis and to validate potential sampling protocols using measurements of breadfruit productivity.

Materials and methods

Foliar sampling protocol development. Nine trees were sampled at the University of Hawai'i at Mānoa campus, Magoon Research Station, and the Lyon Arboretum in Mānoa, HI. Annual average rainfall of the tree locations ranged from 900 to 3700 mm, and soils were Inceptisols with pH values ranging from 5.8 to 6.7. Trees were of various ages and the diameter at breast height of the trees ranged from 12 to 40 cm. Three varieties were represented—Ma'afala, Hawaiian, and Maopo. The management of the trees varied with respect to pruning (from heavy to none) and fertilization (from moderate to none). The selection of trees intentionally spanned a range of environments,

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1	ppm	$mg \cdot kg^{-1}$	1
2.2417	ton(s)/acre	$t \cdot ha^{-1}$	0.4461
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

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management practices, and varieties in an effort to maximize between-tree variance of foliar nutrient levels.

Following previous studies on related species, sun-exposed leaves were collected from the midcanopy (Jones, 2001; Sun et al., 2015). Samples were collected exclusively in Apr. 2016. Although foliar nutrients of tree crops vary considerably throughout the year, previous work on the closely related jackfruit (*Artocarpus heterophyllus*) demonstrated that April measurements most closely approximate the annual average (Sun et al., 2015)—a standard that is applied to foliar nutrient sampling in a range of tree crops (e.g., Bell and Ward, 1984; Dhandhar and Bhargava, 1993; Mahesh and Singh, 2005; Perica, 2001; Zatylny and St. Pierre, 2006). Furthermore, in Hawai'i, April represents the end of the growing flush immediately before the flowering season that typically occurs in May. At this time, vegetative growth is generally slow and neither flowers nor fruit have yet developed.

Three branches from each of the four cardinal directions were selected from each tree. One leaf was collected from the first four leaf positions (leaf position one = first fully emerged leaf at branch tip) on each branch, for a total of 12 leaves for each leaf position per tree. The leaves were immediately taken to the laboratory and rinsed with deionized water. Leaves were divided into multiple parts for analysis: leaf tip (foremost leaf lobe), leaf midrib, petiole, and leaf hole punches from the leaf tissue that avoided major veins. All foliar samples were dried at 40 °C for 72 h, ground with a Wiley mill through a 0.25-mm mesh screen, and analyzed at Brookside Laboratory (New Bremen, OH) for nutrient concentrations. Total concentrations were reported in either percent or parts per million for nitrogen (N), phosphorus, magnesium, potassium, calcium, sulfur, boron, iron, manganese, copper, zinc, and aluminum. For all elements except N, ground leaves were digested in a closed synthetic fluoropolymer (Teflon; Chemours, Wilmington, DE) vessel with nitric acid and hydrogen peroxide using a microwave digester (Mars Microwave; CEM Corp., Matthews, NC) and analyzed on an inductively coupled plasma optimal emission spectrometer (6500 Duo; Thermo Fisher Scientific, Waltham, MA) using method P-4.30 in Gavlak et al. (2003). For N, total combustion using a combustion analyzer (EL Cube C/N; Elementar, Langenselbold, Germany) was conducted following method P2.02 in Gavlak et al. (2003). Standards were employed using tomato leaves from the National Institute of Standards and Technology.

All data analysis was conducted in JMP (version 14.0; SAS Institute, Cary, NC). Summary statistics of mean, range, SD, and CV were derived by treatment. A restricted maximum likelihood model was run for each of the 10 elements with trees as a random-effect variable and leaf position and leaf part as fixed-effect variables. Heterogeneity of variance tests [based on an analysis of means for variances method (Wludyka and Sa, 2004)] were performed using crossed effects of leaf position and leaf part by individual tree to examine the variance of each nutrient concentration within each tree. A second set of heterogeneity of variance tests was conducted using pooled data from all trees to examine the variance of each nutrient concentration across trees.

PROTOCOL VALIDATION AND APPLICATION. A subsequent study conducted foliar sampling of 595 breadfruit trees at 87 orchard sites in Hawai'i (Table 1). Sampling occurred in Apr. 2016, 2017, and 2018. Three to five trees were sampled for each variety at every site. For each tree, four sun-exposed leaves (one leaf from each of the four cardinal directions) in the third position were composited into a single sample. From each leaf, the petiole and 24 leaf punches that avoided major veins were collected. Samples were prepared and analyzed as described previously.

We created a categorical assessment of potential productivity as follows. We measured chlorophyll using a chlorophyll meter (SPAD 502 Plus; Spectrum Technologies, Aurora, IL) and photosynthetic yield using a plant photosynthetic meter (PPM 300; Environmental Analysis and Remote Sensing Earth Environmental Monitoring, Delft, The Netherlands) to assess the density and efficacy of chlorophyll in the leaves. For each tree, 100 measurements were made: 10 measurements each on 10 canopy leaves. Values of chlorophyll and photosynthesis were normalized, averaged, and rounded to create a categorical variable of potential productivity ranging from 0 to 10 using the following equation:

$$ROUND\left\{\frac{\left(\frac{SPAD_{measured}-SPAD_{min}}{SPAD_{max}-SPAD_{min}}\right)+\left(\frac{PHOTO_{measured}-PHOTO_{min}}{PHOTO_{max}-PHOTO_{min}}\right)}{2}\right\}\times 10$$

This relative measure of potential productivity was validated with yield data. For some producer sites (n = 37), yields were either reported by the producers (n = 13) or estimated by the researchers using fruit counts throughout the year (n = 24). Yields ranged from 50 to 500 lb and data were binned into increments of 50 lb. A χ^2 contingency test indicated that yields strongly related to our

Table 1. Summary of sites used for validation of foliar nutrient sampling methods of breadfruit in Hawai'i.

Group description	Sites (no.)	Trees (no.)	Varieties present	Potential productivity[z]	Yield[z]
Statewide commercial orchards	68	294	Hawaiian, Ma'afala, Pūou	Yes	Yes
Big Island commercial producers	9	27	Ma'afala	Yes	Yes
Statewide variety trial	8	234	Hawaiian, Ma'afala, Fiti, Otea, Pua'a	Yes	No
Nutrient deficiency trial	1	30	Hawaiian, Ma'afala, Fiti	Yes	No
Diurnal physiology study	1	10	Hawaiian, Ma'afala	Yes	No
Total	87	595			

[z]Indicates if data were collected.

assessment of potential productivity [r^2 0.91 ($P < 0.0001$)].

We used analysis of variance (ANOVA) to examine whether nutrient concentrations differed significantly between categories of productivity and applied comparative means tests (both the Student's *t* test and Tukey's honest significant difference test) to assess statistical differences between categories. Mean, minimum, maximum, and SD were computed for nutrient concentrations in each category of productivity. Set multiples (4/3 and 8/3) of the SD were applied to compute ranges of nutrient concentrations following previous protocols (e.g., Anjaneyulu, 2007; Sun et al., 2015).

Results and discussion

Effect of leaf position and leaf part on nutrient concentrations. A total of 432 leaves (9 trees × 3 leaves/direction × 4 directions × 4 leaf positions) were sampled, dissected into four parts (leaf tip, midrib, petiole, and leaf punches), and analyzed for nutrient concentrations (Table 2). Restricted maximum likelihood variance components indicated that the within-tree variation (corrected random variable effect) represented only 0.2% to 2.5% of the total variance for each of the elements. Furthermore, the effects test indicate that leaf position, leaf part, and the interaction between leaf position and leaf part were all highly significant drivers of nutrient concentration for all elements. The heterogeneity of variance tests indicated that

Table 2. Average foliar nutrient concentration and CV for four leaf positions and four leaf parts using 432 leaves from nine breadfruit trees from differing environmental and management conditions at Mānoa, HI.

Sampling type	Element[z]	Leaf position no.[y] 1 Mean	1 CV	2 Mean	2 CV	3 Mean	3 CV	4 Mean	4 CV
Leaf punches	Nitrogen (%)	2.72	13.024	2.54	6.104	2.65	5.906	2.80	6.944
	Phosphorus (%)	0.34	17.205	0.23	9.81	0.22	12.03	0.25	9.73
	Magnesium (%)	0.50	7.2452	0.56	14.11	0.54	10.03	0.56	13.58
	Potassium (%)	2.32	9.4619	1.42	7.314	1.17	8.053	1.16	15.25
	Calcium (%)	1.08	6.5086	1.58	6.604	1.72	6.369	2.22	8.705
	Boron (ppm)	41.47	8.7931	79.91	8.019	97.13	11.23	109.72	17.99
	Iron (ppm)	88.65	13.578	73.35	15.01	64.46	11.69	58.26	5.409
	Manganese (ppm)	20.51	8.5756	22.56	6.589	21.83	11.45	21.77	10.52
	Copper (ppm)	9.21	9.1539	7.01	12.41	6.10	13.98	5.54	10.01
	Zinc (ppm)	22.85	22.765	44.56	33.45	16.41	11.06	17.14	9.654
Leaf tip	Nitrogen (%)	3.16	15.962	2.87	16.75	2.96	11.62	3.16	7.222
	Phosphorus (%)	0.31	15.975	0.21	8.106	0.21	5.292	0.21	5.074
	Magnesium (%)	0.47	7.1275	0.51	17.35	0.50	7.509	0.54	12.78
	Potassium (%)	1.94	6.9804	1.30	5.712	1.12	9.608	1.08	11.14
	Calcium (%)	0.97	10.492	1.53	13.32	1.77	3.112	2.02	9.385
	Boron (ppm)	46.25	12.943	91.37	3.404	129.14	15.94	147.76	21.08
	Iron (ppm)	213.08	36.519	124.61	16.37	116.19	21.54	108.44	20.84
	Manganese (ppm)	22.85	9.1287	23.18	10.95	22.78	13.38	22.59	13.22
	Copper (ppm)	11.84	20.068	9.31	23.7	8.04	17.41	11.23	31.55
	Zinc (ppm)	55.47	30.691	47.34	41.43	31.74	20.58	24.04	25.42
Midrib	Nitrogen (%)	1.47	19.195	1.18	27.31	1.21	21.21	1.24	29.66
	Phosphorus (%)	0.33	8.091	0.38	13.61	0.46	9.253	0.58	16.73
	Magnesium (%)	0.70	16.545	0.71	12.59	0.77	20.54	0.96	16.43
	Potassium (%)	3.34	8.5702	3.11	6.838	2.67	9.589	2.03	10.17
	Calcium (%)	1.53	6.0969	1.69	6.125	2.30	6.727	2.96	14.27
	Boron (ppm)	40.01	4.2748	42.90	3.417	43.13	8.431	39.51	2.987
	Iron (ppm)	112.62	34.496	59.24	12.23	58.18	4.662	61.97	17.14
	Manganese (ppm)	11.39	10.174	12.44	18.21	16.85	16.83	15.00	13.97
	Copper (ppm)	7.13	20.483	3.53	16.19	3.58	16.42	4.85	27.87
	Zinc (ppm)	15.43	19.822	15.56	29.43	14.90	15.82	21.69	37.21
Petiole	Nitrogen (%)	0.72	25.639	0.76	23.49	0.91	8.24	0.90	9.613
	Phosphorus (%)	0.42	40.799	0.57	46.09	0.57	15.17	0.57	15.3
	Magnesium (%)	0.57	46.276	0.72	28.89	0.71	23.12	0.73	22.54
	Potassium (%)	2.97	29.218	3.48	20.42	3.97	16.47	3.93	21.62
	Calcium (%)	1.55	35.535	1.91	32.05	2.12	15.17	2.35	30.35
	Boron (ppm)	24.02	18.983	27.15	18.94	30.41	3.387	30.48	3.629
	Iron (ppm)	15.80	24.265	18.81	24.4	16.79	17.22	16.56	18.4
	Manganese (ppm)	11.10	13.784	14.40	19.15	11.28	13.06	11.56	12.58
	Copper (ppm)	2.02	35.132	3.33	52.84	2.87	40.22	2.95	38.83
	Zinc (ppm)	34.16	25.806	31.17	20.38	40.51	28.34	41.77	30.42

[z]1 ppm = 1 mg·kg^{-1}.
[y]Leaf position 1 = first fully open leaf at the branch tip.

Table 3. Element concentrations in foliar samples of breadfruit trees from 595 trees representing 87 orchards in Hawai'i using leaf punches and petioles grouped by categories of productivity: low (n = 75), medium (n = 423), and high (n = 97).

Sample type	Element[z]	All			Low			Medium			High		
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Leaf	Nitrogen (%)	1.19	2.68	4.17	1.19	2.17	3.03	1.54	2.57	3.56	2.60	3.37	4.17
	Phosphorous (%)	0.08	0.21	0.36	0.08	0.18	0.28	0.13	0.22	0.36	0.13	0.22	0.32
	Magnesium (%)	0.13	0.46	0.94	0.13	0.43	0.94	0.25	0.47	0.80	0.17	0.45	0.77
	Potassium (%)	0.72	1.72	2.90	1.36	1.92	2.44	0.73	1.72	2.90	0.72	1.62	2.20
	Calcium (%)	0.38	1.61	2.73	0.38	1.19	2.17	0.91	1.72	2.73	1.02	1.51	2.27
	Sulfur (%)	0.10	0.21	0.31	0.10	0.19	0.27	0.12	0.21	0.31	0.17	0.23	0.30
	Boron (ppm)	12.10	38.54	96.00	16.90	35.40	96.00	12.10	41.48	82.80	16.90	30.71	47.00
	Iron (ppm)	28.90	89.04	310.00	35.30	76.64	211.00	28.90	90.51	310.00	45.20	92.22	217.00
	Manganese (ppm)	11.50	31.08	124.00	11.50	31.63	124.00	11.60	30.21	76.30	12.30	33.67	63.30
	Copper (ppm)	0.90	6.43	17.10	2.00	5.44	12.40	0.90	6.13	12.10	4.40	8.12	17.10
	Zinc (ppm)	8.40	16.14	32.50	9.80	17.18	32.50	8.40	15.96	31.80	10.80	16.03	23.80
	Aluminum (ppm)	1.20	45.80	467.00	7.80	69.40	467.00	1.20	46.66	274.00	11.60	26.62	75.00
Petiole	Nitrogen (%)	0.57	1.03	1.88	0.62	0.73	0.86	0.57	1.00	1.49	0.89	1.29	1.88
	Phosphorous (%)	0.05	0.40	1.07	0.27	0.59	1.07	0.09	0.44	0.90	0.05	0.21	0.42
	Magnesium (%)	0.14	0.77	1.36	0.14	0.67	1.24	0.38	0.87	1.36	0.32	0.61	0.99
	Potassium (%)	1.39	3.87	6.83	3.42	4.95	6.83	1.39	3.63	6.38	2.32	3.69	5.15
	Calcium (%)	0.62	1.97	3.95	0.62	1.17	2.01	0.66	2.22	3.95	1.07	1.93	2.84
	Sulfur (%)	0.03	0.08	0.26	0.04	0.10	0.22	0.03	0.09	0.26	0.03	0.06	0.09
	Boron (ppm)	18.30	30.38	48.00	25.10	31.56	39.80	18.30	31.09	48.00	22.60	28.09	35.40
	Iron (ppm)	9.30	36.32	230.00	9.30	24.06	121.00	11.60	43.98	230.00	13.40	27.86	54.40
	Manganese (ppm)	5.90	17.68	48.20	7.80	19.13	48.20	6.20	17.79	40.60	5.90	16.50	30.40
	Copper (ppm)	0.50	2.70	9.00	0.50	1.51	3.30	0.50	2.92	9.00	0.90	2.98	4.70
	Zinc (ppm)	5.90	31.55	87.00	10.30	36.95	74.80	6.10	32.69	87.00	5.90	25.63	56.00
	Aluminum (ppm)	2.60	29.39	171.00	2.60	19.72	160.00	3.80	38.19	171.00	4.60	18.72	71.80

[z]1 ppm = 1 mg·kg^{-1}.
Min = minimum; Max = maximum.

Table 4. One-way analyses of variance testing differences between nutrient concentrations and categorical groups of "low," "medium," and "high" productivity for leaf (n = 595) and petiole (n = 276) samples of breadfruit trees from 87 orchards in Hawai'i.

Element[z]	Leaf		Petiole	
	r^2	P	r^2	P
Nitrogen (%)	0.4	0.0001	0.45	0.0001
Phosphorous (%)	0.07	0.0001	0.39	0.0001
Magnesium (%)	0.01	0.031	0.21	0.0001
Potassium (%)	0.04	0.0001	0.18	0.0001
Calcium (%)	0.16	0.0001	0.24	0.0001
Sulfur (%)	0.08	0.0001	0.1	0.0001
Boron (ppm)	0.1	0.0001	0.08	0.0001
Iron (ppm)	0.01	0.0166	0.08	0.0001
Manganese (ppm)	0.01	0.0383	NS	NS
Copper (ppm)	0.15	0.0001	0.22	0.0001
Zinc (ppm)	0.01	0.0346	0.05	0.0014
Aluminum (ppm)	0.06	0.0001	0.09	0.0001

[z]1 ppm = 1 mg·kg^{-1}.
NS = not significant.

the third fully open leaf from the branch tip had the lowest within-tree variation for all elements. The low within-tree variation associated with the third leaf matched our anecdotal observations; the first leaf, although fully opened, typically varied in stage of physiological development and the fourth leaf often showed signs of senescence. Thus, leaf positions two or three would likely be the most stable in terms of physiological development and therefore nutrient concentrations. The between-tree variance in element concentrations associated with leaf position did not display any consistent effects.

Different leaf parts had significantly different concentrations of nutrients (Table 2). The use of leaf punches resulted in the lowest within-tree variance, as indicated by heterogeneity of variance tests of each element. However, using the pooled data from all trees, we found the use of petioles resulted in the greatest between-tree variance for most elements. This provides evidence that the nutrient concentrations of the petioles may be more responsive to differences across influential factors such as climate and management.

Relationship of foliar nutrient concentrations to productivity. Leaf punches and petioles from the third mature leaf were selected for further investigation based on our

Table 5. Nutrient concentration ranges in foliar sampling of breadfruit leaves (n = 117) and petioles (n = 73) derived from SD multiples (4/3 and 8/3) from analyses of trees categorized as "high" potential productivity in orchards in Hawai'i.

			Low		Optimal		High		
	Element[z]	Deficient	Min	Max	Min	Max	Min	Max	Excess
Leaf	Nitrogen (%)	<2.30	2.30	2.83	2.83	3.90	3.90	4.43	>4.43
	Phosphorous (%)	<0.07	0.07	0.15	0.15	0.29	0.29	0.36	>0.36
	Magnesium (%)	<0.09	0.09	0.27	0.27	0.63	0.63	0.81	>0.81
	Potassium (%)	<0.70	0.70	1.16	1.16	2.08	2.08	2.54	>2.54
	Calcium (%)	<0.61	0.61	1.06	1.06	1.97	1.97	2.42	>2.42
	Sulfur (%)	<0.14	0.14	0.19	0.19	0.28	0.28	0.32	>0.32
	Boron (ppm)	<11.41	11.41	21.06	21.06	40.36	40.36	50.00	>50.00
	Iron (ppm)	<11.49	11.49	51.85	51.85	132.59	132.59	172.96	>172.96
	Manganese (ppm)	<1.68	1.68	17.67	17.67	49.66	49.66	65.65	>65.65
	Copper (ppm)	<0.91	0.91	4.51	4.51	11.73	11.73	15.34	>15.34
	Zinc (ppm)	<8.50	8.50	12.26	12.26	19.80	19.80	23.57	>23.57
	Aluminum (ppm)	—	0.00	9.22	9.22	44.03	44.03	61.43	>61.43
Petiole	Nitrogen (%)	<0.62	0.62	0.95	0.95	1.62	1.62	1.95	>1.95
	Phosphorous (%)	—	0.00	0.09	0.09	0.33	0.33	0.45	>0.45
	Magnesium (%)	<0.10	0.10	0.36	0.36	0.87	0.87	1.13	>1.13
	Potassium (%)	<1.68	1.68	2.68	2.68	4.69	4.69	5.69	>5.69
	Calcium (%)	<0.71	0.71	1.32	1.32	2.54	2.54	3.16	>3.16
	Sulfur (%)	<0.01	0.01	0.03	0.03	0.08	0.08	0.11	>0.11
	Boron (ppm)	<20.68	20.68	24.39	24.39	31.80	31.80	35.51	>35.51
	Iron (ppm)	—	0.00	13.79	13.79	41.93	41.93	56.00	>56.00
	Manganese (ppm)	<1.12	1.12	8.81	8.81	24.19	24.19	31.88	>31.88
	Copper (ppm)	<0.65	0.65	1.82	1.82	4.15	4.15	5.32	>5.32
	Zinc (ppm)	—	0.00	6.84	6.84	44.41	44.41	63.20	>63.20
	Aluminum (ppm)	—	—	—	0.00	39.89	39.89	61.06	>61.06

[z]1 ppm = 1 mg·kg^{-1}.
Min = minimum, Max = maximum.

analysis of leaf sampling protocols. Composite samples from 595 trees representing 87 sites were analyzed (Table 1). All trees were assessed for potential productivity, and 37 sites were assessed for yields as described in the Materials and Methods. We acknowledge that due to the estimation techniques used in determining yield and the translation of leaf-based measurements to yield that interpolation issues may be present that could influence the outcomes of this study.

We conducted a preliminary assessment of the relationship between macronutrients (N, phosphorus, potassium) and potential productivity. *T*-test analyses of comparative means indicated that productivity category 10 had significantly different nutrient concentrations than all other categories of productivity for all elements, whereas categories 2 to 5 and 6 to 9 tended to cluster into groups that were statistically indistinguishable. On the basis of this initial analysis, we simplified our productivity categories used in subsequent analyses to be "low" (2–5), "medium" (6–9), and "high" (10).

The average and range of foliar nutrient concentrations are presented (Table 3). ANOVA indicated that both leaf punches and petioles demonstrated highly significant differences in nutrient concentrations when grouped by "low," "medium," and "high" categories of productivity (Table 4). However, the petioles demonstrated much stronger differences in terms of r^2 values (Table 4).

We developed nutrient standards based on trees only from the "high" productivity category. Following previous protocols (e.g., Anjaneyulu, 2007; Sun et al., 2015) we used multiples (4/3 and 8/3) of the SD to define five categories (deficient, low, optimal, high, excessive) of nutrients (Table 5). We emphasize that this method only provides a general overview of nutrient ranges and is not empirically derived optimal nutrient ranges. However, due to the current lack of information on breadfruit tree nutrition, we present these figures for consideration by growers and researchers.

As a validation of these proposed ranges, we used data from 68 production sites to examine the relationship between chlorophyll and N concentration in the leaf punches (Fig. 1). Previous research suggests that across a broad range of tree crops the relationship between chlorophyll and N concentrations is linear (Chang and Robison, 2003; Neilsen et al., 1995; Netto et al., 2005; Van den Berg and Perkins, 2004). However, we observed an asymptotic relationship. Anecdotally, the relationship illustrates that at values greater than ≈2.75% N (Fig. 1B) chlorophyll meter readings are high (≈57) and stable; between ≈2.35% and ≈2.75% N (Fig. 1A) chlorophyll meter readings are high (≈55) but moderately variable; and less than ≈2.35% N (Fig. 1C) chlorophyll meter readings drop off precipitously with decreasing N. The outlying sites (Fig. 1D) had very high concentrations of manganese and aluminum, and we attributed their position to toxicity. The concentrations of N delineated in Fig. 1 are reasonably aligned with the ranges provided in Table 5, approximating the minimal optimum range (2.83%), and the upper end of deficient (2.30%). Alternatively,

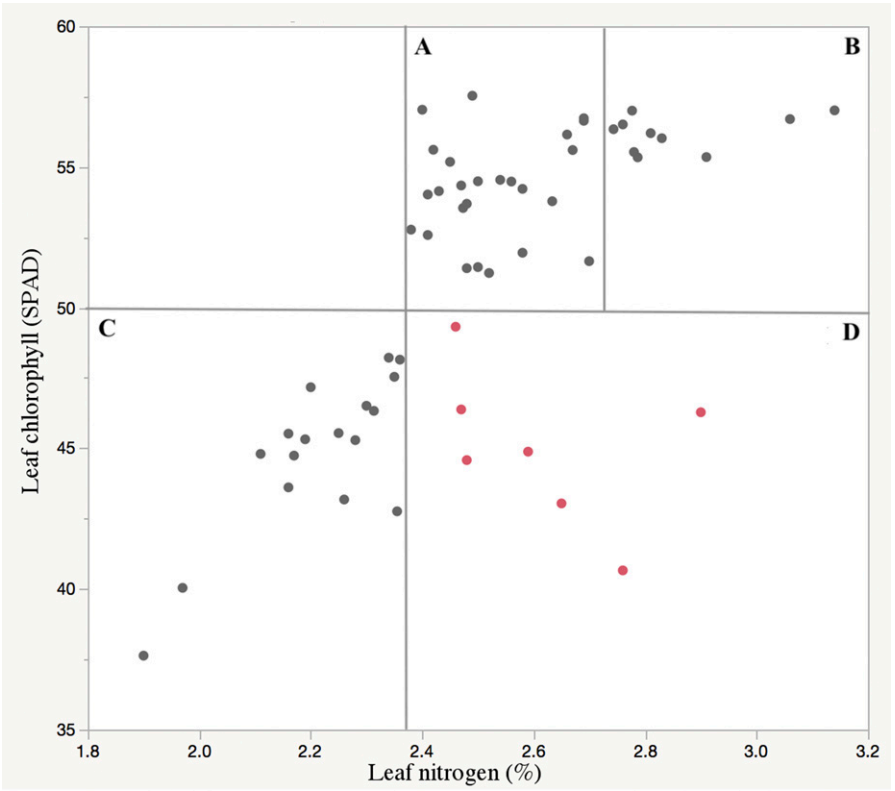

Fig. 1. Relationship between leaf chlorophyll and foliar nitrogen in leaf punches of breadfruit trees from 68 producer sites in Hawai'i, illustrating chlorophyll meter levels that are high and stable in (B), high but variable in (A), and steeply declining in (C). Box (D) had high concentrations of manganese and aluminum and we attributed their position to toxicity.

applying the Mitscherlich equation (e.g., Gomes, 1953; Ware et al., 1982) to this dataset suggests a critical N level in the leaf punches of 2.35% associated with 95% of maximum chlorophyll levels. We suggest that the standards presented are a reasonable portrayal of appropriate foliar nutrient levels, although more research is needed to refine these figures.

Conclusion

We established that the third mature leaf from the stem tip produced the least variable within-tree measurement of nutrient concentrations. We demonstrated that using leaf punches produced the least variable within-tree measurement and that petioles were most responsive to changes across sites. We used orchard sites to examine the relationships between foliar nutrients and potential production and used limited yield data to validate a measure of productivity. We observed that both leaf punches and petioles were significantly related to productivity but that the petioles demonstrated stronger differences in nutrient concentrations.

We applied multiples of the SD of foliar nutrient concentrations of trees in the "high" category of productivity to provide preliminary foliar nutrient recommendations. We then verified that the recommendations for N reasonably aligned with physiological performance in terms of chlorophyll measurements. In conclusion, we advocate that these sampling protocols be used by breadfruit producers and researchers more broadly to produce comparable results and further refine the recommendations for breadfruit sampling for nutrient analyses.

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