

A comparison of diagnostic sampling methods in bananas.

J. H. L. MESSING*

UNA COMPARACIÓN DE MÉTODOS DE MUESTREO
DIAGNOSTICAL EN PLÁTANOS (BANANOS)

UNE COMPARAISON DE METHODES D'ECHANTILLONNAGE
DIAGNOSTICIEL SUR BANANIERS

J.H.L. MESSING

Fruits, mars 1978, vol. 33, n°3, p. 167-181.

RESUME - Vingt bananeraies ont été échantillonnées dans les Iles du Vent, en utilisant simultanément quatorze méthodes différentes de prélèvement qui comprenaient deux stades de croissance de la plante, différents âges de la feuille, différentes parties du limbe, le pétiole et la nervure. Les teneurs en N, P, S, K, Ca, Mg et Mn des échantillons ont été déterminées.

Les résultats ont été comparés à l'aide d'une analyse en composantes principales. L'accord entre les résultats obtenus sur les divers modes de prélèvement a été mauvais pour P, satisfaisant pour N, S, K et Ca, bon pour Mg et excellent pour Mn. Toute la divergence relevée est apparue physiologiquement acceptable.

Au cours de cette étude, aucun type d'échantillon ne s'est montré clairement préférable à la partie centrale du limbe de la feuille III, largement utilisée.

INTRODUCTION

MARTIN-PRÉVEL (1974) published the results of a questionnaire circulated to most of the laboratories concerned with foliar analysis of the banana plant. Methods used for sampling the plants differ considerably from place to place and he proposed a study of a group of selected methods with the object ultimately of establishing an international reference method (Méthode d'Échantillonnage internationale de Référence - M.E.I.R.). MARTIN-PRÉVEL

pointed out the benefits which would result from such action.

At the Winban Research and Development Centre, leaf analysis has been used during the past nine years both in support of experimental work with bananas and in advice offered to banana growers. A large volume of data and experience has accumulated.

At the beginning the Winban standard sample was taken from large (full height) unshot plants (one to two months before shooting). The lamina of leaf IV was used counting the youngest emerging leaf as in position I as soon as it started unrolling (TWYFORD and COULTER, 1964). In practice this meant that, depending on the state of the youngest leaf, the sampled leaf was the third or occasionally the fourth fully open leaf. To define the position more precisely and to conform with the majority of other

* - Research and Development Division Windward Islands Banana Grower's Association, P.O.B. 115 - CASTRIES, Santa Lucia
Communication présentée au Premier Séminaire international sur l'Analyse foliaire du bananier, Ténérife, août 1975.

workers, from mid-1971, samples were taken from leaf III as the third fully open leaf. This change did not produce significant difference in values and no adjustment was necessary. All the data presented in this paper, and referred to as «Standard», represent two strips 5 to 10 cm wide taken from both side of the leaf III at its centre.

METHODS

To compare the values obtained by the Winban standard method of sampling with those obtained when alternative methods are used, 20 plantations (5 in each island) were sampled during December 1974 and January 1975. In each place samples were taken simultaneously by the standard method and using 13 other methods, most of them suggested by MARTIN-PRÉVEL (1974) for an international investigation. A list of the places, dates of sampling, variety and crop cycle are given in Table 1.

Figure 1 illustrates all the fourteen methods of sampling used at each site.

For sampling methods 2 to 7 inclusive, the same «large» stage of growth of the plant was chosen (after floral differentiation). The strips similar to those taken for standard sample, were taken from the adjacent location on the same leaf and halved. The inner halves constituted sample 2 and the outer sample 3. Methods 4 and 5 were comparable inner and outer lamina halves respectively but taken from the first fully open leaf. Samples 6 and 7 comprised a central portion of petiole (about 5 to 10 cm long) and a central portion of midrib (about 15 cm) respectively, taken from the oldest surviving leaf (more than 50 % of the surface still green).

For sampling methods 8 to 14 inclusive, the stage of growth was shot, with the first male hand just exposed. Discounting the spade leaf, methods 8 to 10 were taken from the third youngest leaf and consisted of samples cognate to methods 1, 2 and 3 respectively and 11 was a central part (ca. 15 cm) of the midrib of the same leaf. Finally, now counting the spade leaf as the first, methods 12, 13 and 14 were equivalent to 9, 10 and 11 respectively on the third youngest leaf. Thus, methods 8 to 11 were

TABLE 1 - List of sampled sites.
TABLEAU 1 - Liste des emplacements échantillonnés
CUADRO 1 - Lista de los sitios muestrados

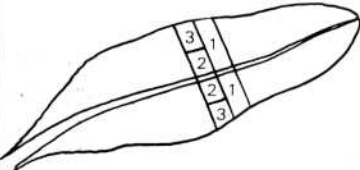
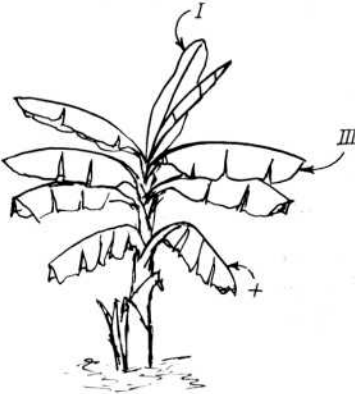
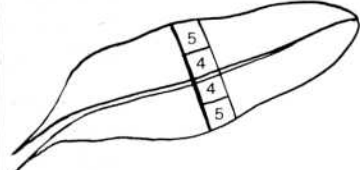
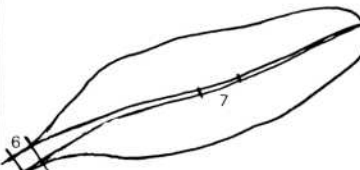
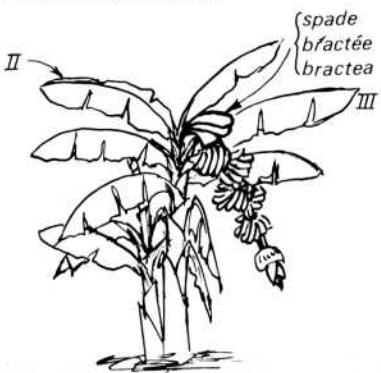
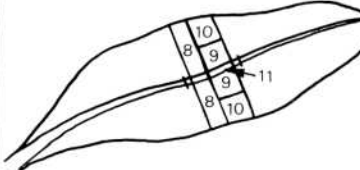
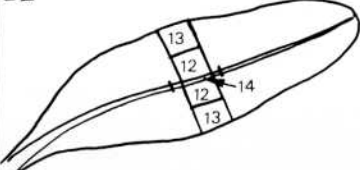
No	Island Ile Isle	Date Date Fecha	Location Lieu Lugar	Variety Cultivar Variedad	Crop cycle Cycle de culture (*) Ciclo de cultivo
1	Grenada	7/01/75	Mirabeau Estate	'Robusta'	1° R
2		7/01/75	Pachier	'Robusta'	1° R
3		8/01/75	Dougalstone Estate	'Robusta'	1° R
4		8/01/75	Plaisance	'Robusta'	Plt
5		9/01/75	Capitol Estate	'Giant Cavendish'	Plt
6	St Vincent	10/12/74	Wallilabou Estate	'Robusta'	1° R
7		11/12/74	Orange Hill Estate	'Valery'	1° R
8		11/12/74	Mt. Bentick Estate	'Lacatan'/'Robusta'	X° R
9		12/12/74	Montreal	'Grand Naine'	Plt
10		12/12/74	Dumbarton	'Lacatan'/'Robusta'	X° R
11	St Lucia	27/11/74	Forestierre	'Robusta'	1° R
12		28/11/74	Roseau, exp. Farm	'Valery'	1° R
13		5/12/74	Marquis Estate	'Valery'	1° R
14		19/12/74	Dennerly	'Robusta'	1° R
15		19/12/74	Cul-de-Sac Estate	'Robusta'	1° R
16	Dominica	28/01/75	Giroudel	'Robusta'	Plt
17		28/01/75	Layou Valley	'Robusta'	Plt
18		29/01/75	Rosalie Estate	'Robusta'	1° R
19		29/01/75	Melville Hall	'Robusta'	Plt
20		29/01/75	Woodford Hill Est.	'Giant Cavendish'	1° R

(*) - Plt : plant crop - 1er cycle («planté») - 1er ciclo («plantado»)

1° R : 1st. ratoon - 2e cycle (1er rejeton) - 2 ciclo (1er retoño)

X° R : older ratoon - neme cycle (n-1ème rejeton) - n° ciclo (n-1° retoño).

Figure 1 • SAMPLING METHODS - METHODES D'ECHANTILLONNAGE - MÉTODOS DE MUESTREO.

STAGE - STADE - FASE	POSITION	METHOD N°
<p>Unshot.- Non fleuri - <i>Sin flor</i></p> <p>≤ 6 weeks before shooting. ≤ 6 semaines avant floraison. ≤ 6 sem. antes de floración.</p>	<p>III</p> 	<p>1. Standard 2. Intern 3. Extern</p>
	<p>I = Youngest fully open. Plus jeune entièrement déroulée. Más joven de las hojas desevolvidas.</p> 	<p>4. Intern 5. Extern</p>
	<p>+ = Oldest surviving. Plus âgée des feuilles vivantes. Más vieja de las hojas vivas.</p> 	<p>6. Petiole 7. Midrib-Nervure-Nervio</p>
<p>Shot. - Fleuri - <i>Con flor</i></p> <p>1st ♂ hand uncovered. 1^{re} main ♂ découverte. 1^a mano ♂ descubierta.</p> 	<p>III Spade counted as 0. Bractée comptée 0. Bractea contada como 0.</p> 	<p>8. Complete strips Bandes entières Fajas enteras 9. Intern 10. Extern 11. Midrib-Nervure-Nervio</p>
	<p>III Spade counted as I. Bractée comptée I. Bractea contada como I. = II</p> 	<p>12. Intern 13. Extern 14. Midrib-Nervio-Nervio</p>

taken from the third fully-sized leaf and 12 to 14 from the second full-sized leaf.

All samples were taken simultaneously by first locating a shot plant with the first male hand uncovered and sampling it using methods 8 to 14. Then the nearest unshot plant at the correct stage was sampled by methods 1 to 7. The procedure was repeated until sufficient pairs of plants were sampled to obtain enough plant material for analysis. This technique ensured that conditions represented by all fourteen samples were as closely similar as possible.

The samples were dried at 80°C, ground in a Glen-Creston micro-hammer mill and analysed by the routine methods used at Winban Research and Development Centre for N, P, S, K, Ca, Mg and Mn.

Total N was determined by distilling the ammonia from a concentrated H₂SO₄/H₂O₂ digest of the plant material into standard boric acid using Markham apparatus.

Total P was determined in an aliquot of the same digest using the Molybdenum Blue method.

For M, Ca, Mg and Mn a sample was ignited at 550°C and the ash dissolved in dilute HCl. K was determined by flame photometry and Ca, Mg and Mn using atomic absorption.

RESULTS

The results of analysis for N, P, S, K, Ca, Mg and Mn are summarised in Table 2.

The results for each nutrient were analysed statistically by the technique of principal component analysis. The coefficients of the first principal component in each set of data were all positive, which indicates that the general overall level of nutrient in the plants is the effect which caused most of the variation between observations. High values of the percentage of variance «explained» by the general level of the nutrient corresponds to a high level of agreement between the results obtained by the various methods of sampling used. Table 3 gives the percentages of total variance explained by the first principal component for each nutrient, separately for sampling methods from unshot plants and from shot plants. Principal components analysis was performed on three sets of data, first using covariance, secondly using the correlation matrix including both within and between island variation and thirdly with the correlation matrix of within-island pooled variation, after eliminating the between-island variation.

Principal component analysis performed on correlation matrix is preferable to one based on covariance as the

latter exaggerates the importance of the sampling methods holding a greater amount of variability. However a greater proportion of total variance was explained by the principal component when the covariance matrix was used. Table 3 shows that the agreement between various methods of sampling is poor for P, fair for N, S, K and Ca, good for Mg and excellent for Mn.

The next stage of principal component analysis allow us to obtain some information on the cause of the remaining variation after the first principal component has been removed. On examining the value and sign of the coefficients of the second principal component it is quite apparent that for all the nutrients studied there was a contrast between one or two methods on the one hand and a group of two methods on the other hand, both in unshot and shot plants. In the majority of cases this disagreement explains a fair proportion of the remaining variation. The values for each nutrient in the shot and unshot series are given in the last three columns of Table 3.

In unshot plants for N, P and S the contrast was between oldest leaf petiole and midrib on one hand and both parts of the first leaf lamina on the other. A similar contrast was present in the case of Ca, Mg and Mn but for K the contrast was mainly with the outer parts of both first and third leaves.

In the shot plant series, the main contrast for all nutrients was between the midrib of both leaves sampled and the outer parts of one or both laminae. All the contrasts noted seem physiologically acceptable.

A further point of interest in the data of Table 3 is the comparison between N, P and S on the one hand and K, Ca, Mg and Mn on the other.

Using either of the correlation matrices a greater proportion of variation was explained by the general nutrient level for N, P and S in unshot plants but for K, Ca, Mg and Mn in shot plants. On the other hand, the variation due to contrast between methods was in the opposite sense.

DISCUSSION

The survey type data presented in this paper were obtained to facilitate discussion during the proposed seminar on banana leaf sampling standardisation. Principal component analysis provides a good indication of the amount of agreement between the story told by any one sampling method and that told by the other methods. The technique of sampling ensured that the plants used were very closely comparable in field conditions but were differing as far as possible from site to site in geographical position, variety, crop, etc. The main weakness of the survey was that in

TABLE 2 - Summary of the results of analysis of the 20 plantations (expressed on dry matter)
 TABLEAU 2 - Résumé des résultats d'analyses des 20 plantations (exprimés par rapport à la matière sèche)
 CUADRO 2 - Resumen de los resultados de análisis de las 20 fincas (referidos a la materia seca).

Plant stage Stade plante Fase planta	method	N %			P %			S %			K %			Ca %			Mg %			Mn p.p.m.		
	see voir fig. 1 ver	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.	min.	max.	mean moy. promed.
Unshot non fleuri sin flor	1 : standard	2.41	3.37	2.81	.169	.223	.193	.180	.274	.221	3.15	4.75	3.77	.375	.750	.540	.180	.445	.318	135	3000	1087
	2 : III inter.	2.18	3.08	2.59	.147	.211	.182	.168	.250	.199	3.50	5.60	4.33	.345	.810	.534	.135	.458	.303	110	1800	687
	3 : III exter.	2.65	3.70	3.11	.170	.233	.200	.204	.298	.246	2.50	3.85	3.25	.375	.740	.584	.223	.510	.362	200	4280	1602
	4 : I intern.	2.27	2.83	2.49	.183	.283	.242	.164	.216	.184	4.00	5.90	4.94	.145	.500	.302	.182	.356	.284	60	1240	419
	5 : I extern.	2.83	3.61	3.07	.199	.292	.242	.188	.264	.220	2.90	4.60	3.57	.155	.475	.317	.273	.473	.338	110	1970	615
	6 : petiole	.35	.69	.50	.038	.085	.059	.044	.274	.108	.55	7.40	2.98	.525	1.590	.961	.066	.494	.241	125	3000	1086
	7 : mid./ nerv.	.36	.66	.49	.030	.095	.059	.060	.256	.124	.50	5.10	2.27	.575	1.820	.922	.056	.435	.237	125	3000	1152
Shot fleuri con flor	8 : III com.	2.20	3.00	2.54	.145	.204	.178	.176	.264	.201	2.95	4.15	3.44	.455	.945	.656	.193	.384	.303	140	3330	1195
	9 : III int.	2.09	2.76	2.34	.141	.203	.173	.152	.216	.180	3.00	5.15	3.96	.455	1.090	.678	.158	.435	.301	95	2380	840
	10 : III ext.	2.51	3.65	2.88	.172	.215	.195	.194	.274	.222	2.60	3.65	2.96	.455	.890	.644	.276	.445	.328	200	4800	1784
	11 : III mid./ nerv.	.33	.68	.46	.040	.136	.094	.026	.080	.044	1.90	5.90	3.87	.355	1.100	.668	.060	.396	.237	70	1430	583
	12 : II int.	2.07	2.83	2.37	.165	.212	.191	.150	.220	.177	3.20	4.90	3.96	.455	1.000	.655	.162	.392	.296	90	2060	748
	13 : II ext.	2.67	3.60	3.03	.172	.236	.207	.194	.250	.217	2.65	3.45	3.08	.410	.820	.611	.248	.427	.330	145	4280	1508
	14 : II mid./ nerv.	.40	.60	.49	.045	.168	.112	.028	.076	.045	2.05	6.05	4.11	.315	1.165	.653	.086	.384	.241	90	1620	537

TABLE 3 - Percentage of total variance within groups of samples (unshot, shot) explained by 1st and 2nd principal components.

TABLEAU 3 - Pourcentage de la variance totale intra-groupes (non fleuri ou fleuri) expliqué par les 1ère et 2ème composantes principales.

CUADRO 3 - Porcentaje de la variancia total intra-grupos (sin o con flor) explicado por las 1a y 2a componente principal.

Nutrient Elément nutritif Nutriente	Set of samples Groupe d'échantillonnage Grupo de muestras	% due to general level of nutrient (explained by the 1st principal component) % dû au niveau général de l'élément (expliqué par la 1ère composante principale) % debido al nivel general del nutriente (explicado por la 1a componente principal)			Agreement between methods of sampling accord entre méthodes d'échantillonnage acuerdo entre métodos de muestreo	% due to contrast between methods or groups of methods (explained by the 2nd princ. comp.) % dû au contraste entre méthodes ou groupes de méthodes (expliqué par la 2e comp. princ.) % debido al contraste entre métodos o grupos de métodos (explicado por la 2a comp. princ.)		
		within and between islands intra- et inter-îles intra-y inter-islas		within islands pooled correlation matrix matrice de corrélation intra-îles réunies matriz de correlación intra-islas reunidas		within and between islands intra- et inter-îles intra- y inter-islas		-within islands pooled correlation matrix -matrice de corrélation intra-îles réunies -matriz de correlación intra-islas reunidas
		covariance matrix	correlation matrix			covariance matrix	correlation matrix	
N	1 - 7	84.94	74.09	75.99	fair satisfaisant satisfactorio	8.30	16.47	15.31
	8 - 14	85.79	71.06	74.54		6.46	17.79	15.92
P	1 - 7	69.38	69.51	59.64	poor mauvais malo	20.11	17.06	22.92
	8 - 14	63.14	50.83	46.99		25.79	29.96	28.05
S	1 - 7	75.56	69.40	74.59	fair satisfaisant satisfactorio	17.54	18.66	15.32
	8 - 14	67.78	66.67	66.79		15.63	16.97	18.34
K	1 - 7	86.91	63.49	62.67	fair satisfaisant satisfactorio	5.82	17.27	18.73
	8 - 14	85.81	71.61	77.77		7.80	16.48	12.12
Ca	1 - 7	86.35	68.51	67.40	fair satisfaisant satisfactorio	8.79	20.79	19.22
	8 - 14	84.36	81.38	76.04		8.61	9.99	13.50
Mg	1 - 7	80.97	73.06	78.81	good bon bueno	11.22	14.88	11.57
	8 - 14	82.00	80.77	84.90		13.08	13.67	10.69
Mn	1 - 7	95.03	93.33	90.84	excellent	3.42	4.64	6.40
	8 - 14	99.05	98.46	98.12		0.45	0.83	0.97

order to ensure sufficient numbers of plants at the correct stage for sampling use had to be made of larger estates. Most of these estates had been using the Winban Advisory service for a number of years and this limited the range of fertilizer nutrient levels to higher values and thus restricted the value of the survey from a diagnostic point of view.

To provide a visual means of comparison of data for the fourteen methods without giving preference to any one of them, means of all 14 methods were calculated for each nutrient on each site. Using these means, sites were ranked from lowest to highest and divided into three arbitrary groups designated low, medium and high, containing approximately equal number of sites. The division did not have diagnostic significance.

The range of these groups is graphically presented in Figure 2 as solid black blocks. The same groups of sites for all 14 sampling methods are also displayed in the figure.

The discrimination of any one method between the three groups indicates how closely the particular method gives a ranking pattern similar to that of the means.

The close agreement between the conclusions to be drawn from the comparisons and those indicated by the analysis of principal components provides evidence that the means of all fourteen methods form a reasonable basis for the segregation of the sites.

As would be expected from the normal distribution, the range of means in the medium group is smaller than in the low or high groups (except for Mn). This makes for easy merging of this group with the other two. The shading on the diagram indicates the range over which each method failed to discriminate between the low and high groups. Figures immediately below each diagram express the shaded area as a percentage of the total range obtained by that method.

Nitrogen.

Correct diagnosis of nitrogen status in banana plants is of primary importance. Its level influences the yield more often than that of any other nutrient and also because soil analysis does not provide reliable indication of the need to apply it.

The concentration of N was higher in the outer half of the lamina than in the inner half (means for 80 comparisons 3.02% and 2.45% respectively). The difference appeared to be little affected by leaf position, stage of growth or even the level of nitrogen in the plant. Correspondingly, it was found by TWYFORD and COULTER (1964) that nitrogen concentrations were higher in the distal part of the lamina than in the proximal part.

There was less nitrogen in shot plants than in unshot

plants, the reduction being small at the early stage of bunch development sampled.

The concentration of nitrogen in petioles and midribs was comparatively low, about 17% of that in the standard sample and was apparently unaffected by shooting or leaf position. The amount of N present in conductive tissues probably reflected the momentary availability of supply and much less the level in the plant.

Many investigators consider that high concentration of a nutrient in an organ is important when choosing a sample for diagnosis and the high content of N in the third leaf of the banana plant was an important reason for its widespread adoption as a diagnostic sample. It is regrettable that the sample richest in nitrogen has to be discarded in territories where edge scorch occurred. Scorch rarely occurs on the third leaf in the Windward Islands and our standard sample, consisting of whole strips, gives reliable results with a good range of values. In this series, the standard sample most clearly differentiated between high and low values as can be seen in Fig. 2.

Comparison between the first and third leaf is of interest. The range of values recorded was smaller for the first leaf than for the third and the narrowing was due to disappearance of both extremes. This suggests that when there is a shortage of nitrogen the available supply is directed towards the first leaf but when nitrogen is abundant it accumulates more rapidly in the third leaf. It could only be concluded that the N content in the third leaf is more sensitive to the nitrogen supply position than the first leaf.

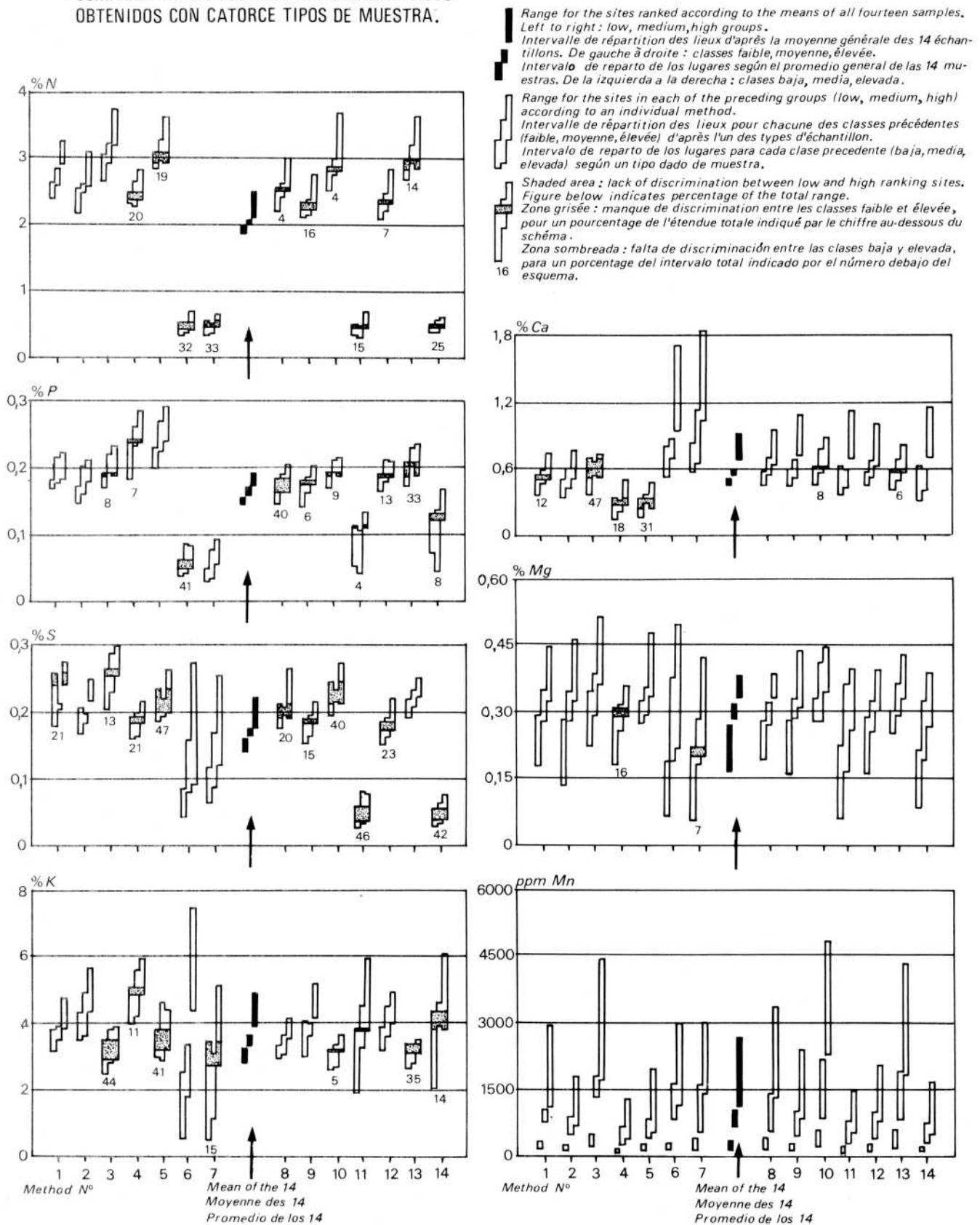
Figure 2 illustrates the above argument and also indicates that the outer part of the lamina together with the Winban standard sample are more sensitive indicators of excessive and thus wasteful, use of nitrogenous fertilizer than the inner parts used in some countries. This is an important feature in view of the high cost of fertilizer.

Phosphorus.

Highest values and the widest range of values were obtained when sampling the first leaf of unshot plants. The outer lamina was richer in P than the inner but the difference was smaller than for N and affected by leaf position, being very small in the younger leaf. Concentration of P was low in the petiole and midrib of the oldest surviving leaf in unshot plants but the comparatively high concentration and the wide range of values in the midrib of the leaves taken from shot plants could make it diagnostically valuable.

Comparison of sampling methods for the diagnosis of P status of the plants is handicapped by the absence of low values which are known from long experience and past work in the Windward Islands to indicate the need for and definite response to applied P.

Figure 2 • COMPARISON OF ANALYTICAL RESULTS OBTAINED FROM FOURTEEN METHODS OF SAMPLING.
 • COMPARAISON DES RESULTATS ANALYTIQUES OBTENUS AVEC QUATORZE TYPES D'ECHANTILLONS.
 • COMPARACIÓN DE LOS RESULTADOS ANALÍTICOS OBTENIDOS CON CATORCE TIPOS DE MUESTRA.



Sulphur.

Sulphur deficiency in bananas was identified in the Windward Islands in 1967 and widespread low values were recorded in a thorough survey of banana plantations carried out in the same year. Correction of the deficiency was easily accomplished and resulted in spectacular improvement of growth. Since then sulphur has been used in all mixed fertilizers at an average content of 4% S.

As in the case of P, the absence of low values from the range obtained in the present study makes comparison of the diagnostic merit of the sampling methods difficult. Most methods, with the exception of the midrib of shot plants, distinguish fairly well between lower and higher values. The oldest surviving leaf petiole and midrib showed some interesting possibility of improving diagnosis (Fig. 2).

The definite drop in S content soon after shooting, more pronounced in older than in younger leaves, would suggest rapid transport of S to sites of greater requirement. The extremely low S content in the midrib of both leaves of shot plants might indicate that the supply was not sufficient at this stage of the plant's life. This could affect fruit quality, as reported by MELIN (1970) and MARCHAL et al (1972).

Potassium.

Potassium is the only nutrient in the group studied which was more concentrated in the inner part of the lamina adjacent to the midrib than the outer part. TWYFORD and COULTER (1964) and later LAHAV (1972) found that potassium ranges from highest in the proximal part of the leaf to lowest in the apex. Petiole and midrib had very variable concentration, very high in some cases and extremely low in others. This is not surprising as potassium is highly mobile inside the plant and does not form any stable chemical combination or accumulate permanently in any organ. It is often highest in conductive tissue. There is little evidence that high or low values found in conductive tissue are related to the general status of this nutrient in the plant. In this survey plants well supplied with this nutrient, judging from their performance and from soil K level showed a range of potassium in the midrib and petiole from very low to very high. It can only be supposed that the values obtained for petiole and midrib represent a very transitory state, strongly affected by factors influencing uptake from hour to hour, e.g. temperatures, soil moisture, time of the day, or alternatively by the need of the plant to redirect potassium to the organ with the greatest current demand, e.g. developing bunch.

On the other hand, the values obtained by analysis of laminae, such as the Winban standard sample, correlate well with plant performance, visual symptoms, soil K supply, application of fertilizers, etc. The data for the third leaf

lamina have long served well as a guide for fertilizer requirements in the Windward Islands and are reliable even in more complicated problems of cation unbalance.

At first sight Fig. 2 suggests that conductive tissue (petiole and midrib) could be valuable for diagnosis. It is however difficult to imagine critical values for these organs considering that plantations having adequate K supply and giving good yields produced values as low as 0.5% of K. There is little room indeed for deficient plants having 2.2% K in leaf III lamina or even less as against a quite adequate level of 3.2%, the lowest value in the present investigation. When statistical examination was performed on the survey figures the coefficient of variation for petioles and midribs was 60 to 70% whereas that for laminae between 5 and 7%. Similar values were found by TWYFORD (private communication)

Calcium.

There is good agreement between all sampling methods in assessing calcium status of the plant. The Winban standard sample agreed closely with the oldest surviving leaf petiole and midrib, which had the widest range in the batch. Inner laminae distinguished better between low and high levels than outer.

The level of calcium in the plants varies with many factors. No clear cut case of calcium deficiency has been observed in the Windward Islands and critical level is thus not strongly established. 0.5% in the standard sample has been used for guidance for many years. More recently, high yields have been achieved mainly due to judicious use of potassic fertilizers. In some of these cases, a calcium level below 0.5% has been noted without any apparent bad effect on yields or quality of bunches, as judged by their appearance.

No deficiency symptoms have been seen and accordingly what appears to be the more realistic level of 0.45% in our standard sample is now considered a safe lower limit for calcium. Even then occasional figure below this value is noted and this often on plantations achieving high yields.

The agreement between the various methods of sampling used in the survey was good, only just inferior to that for Mn and Mg, and as there is no clear evidence that the growth or yield of bananas is affected by calcium nutrition in the Windward Islands consideration of calcium cannot have serious impact on the choice of a diagnostic method, at least for the present.

Magnesium.

The level and range of magnesium present in the standard samples are good and difficult to improve by considering any other tissue. It is however possible that petiole or midrib of some older leaf could prove equally useful. The

principal component analysis clearly indicate that there is little difference between the sampling methods used in this study and whichever method is chosen, the assessment of magnesium status should not present a problem.

The leaf III level of magnesium, used for many years in the Windward Islands, reflects the soil supplying power well, correlates closely with magnesium deficiency symptoms and responds clearly to magnesium supplied in fertilizer. It has been very useful in solving many cases of magnesium deficiency in the area.

Manganese.

Manganese is the nutrient, the supply of which is almost directly reflected in the content found in most tissues of the plant. The banana plant is no exception and in the present study, the percentage of total variation explained by the first principal component was not far from 100%. The results obtained by any one of the sampling methods used correlated very closely with those obtained by any other method and it is safe to say that each method is a good indication of supply. The wide range of manganese availability in Windward Islands, soils helped to produce this very close correlation between all methods of sampling as it covered fairly uniformly the range from just above deficiency almost to toxicity, conditions not easily achieved for any of the major nutrients.

CONCLUSIONS

This study confirms the discriminative value of the whole strips taken from the centre of leaf III lamina as a single diagnostic tissue in bananas. In the survey reported, this sampling method gave a good range of values and discrimination for nitrogen and potassium, the most commonly

required nutrients in banana fertilizers in the Windward Islands.

In the cases of magnesium and manganese all samples seemed highly satisfactory and there was thus no particular superiority of the third leaf lamina.

Phosphorus and sulphur deficiencies are now uncommon in the region. Using the third leaf lamina as a guide nutritionally low values did not arise in the survey data, as expected. It is therefore difficult to be sure of the meaning of low and high values in the third leaf lamina and indeed in any other sample. Nevertheless the results indicate that, while leaf III lamina produced fair discrimination, improvements might be possible. However whereas for P, the outer leaf I lamina (unshot plants) looked promising, this was far from the case for sulphur, where oldest leaf petiole and midrib (unshot plants) seemed preferable. The only similar feature, as indeed also for N, was that unshot plants appeared better than shot.

Calcium is in a different category from any other nutrient in that it has never been proved deficient in the Windward Islands. Critical levels for the leaf III lamina are therefore not well established. Fortunately it appears that most methods give reasonable discrimination over the range encountered except for outer lamina of both leaves I and III in unshot plants.

No clear single alternative to the widely used central part of leaf III lamina emerged from the study.

ACKNOWLEDGEMENT

Grateful acknowledgement is made to Alan BREWER, Caribbean Research and Development Institute, for Statistical analysis and comments on the results.

BIBLIOGRAPHY

1. MARTIN-PREVEL (P.). 1974.
Les méthodes d'échantillonnage pour l'analyse foliaire du bananier.
Résultats d'une enquête internationale et propositions en vue d'une référence commune.
Fruits, 29, 9, 583-588.
2. TWYFORD (I.T.) and COULTER (J.K.). 1964.
Foliar diagnosis in banana fertilizer trials.
in : *Plant Analysis and Fertilizer Problems*, IV, p. 357-370.
3. LAHAV (E.). 1972.
Facteurs influençant la teneur en potassium dans la troisième feuille du rejet de bananier.
Fruits, 27, 9, 585-590.
4. MELIN (Ph.). 1970.
Effets de forts apports minéraux sur le bananier.
Fruits, 25, 11, 763-766.
5. MARCHAL (J.), MARTIN-PREVEL (P.) and MELIN (Ph.). 1972.
Le soufre et le bananier.
Fruits, 27, 3, 167-177.

UNE COMPARAISON DE METHODES D'ECHANTILLONNAGE DIAGNOSTICIEL SUR BANANIERS

INTRODUCTION

MARTIN-PRÉVEL (1974) a souligné la diversité des méthodes en usage pour l'analyse foliaire du bananier et proposé l'étude d'un ensemble de types de prélèvements sélectionnés en vue de parvenir à une méthode internationale de référence.

Le Centre de Recherches et de Développement du Winban (Association des producteurs de bananes des Iles du Vent) a accumulé depuis neuf ans de nombreuses données d'analyse foliaire en appui de l'expérimentation et pour le conseil aux planteurs.

La définition de l'échantillonnage pratiqué au Winban a évolué depuis TWYFORD et COULTER (1964) afin de désigner la position des feuilles selon le même critère que la majorité des autres chercheurs. L'échantillonnage «Standard» représente, dans cet article, deux bandes de 5 à 10 cm de largeur prélevées sur les deux demi-limbes au centre de la feuille en position III.

METHODES

Dans vingt plantations, cinq de chaque île (tableau 1), on a prélevé en décembre 1974 et janvier 1975 l'échantillon «Standard» et 13 autres, la plupart selon les suggestions de MARTIN-PRÉVEL (1974) : voir figure 1. Le stade non fleuri se situe après la différenciation florale. La «plus vieille feuille vivante» a plus de 50 p. 100 de sa surface encore verte.

Pour assurer la meilleure similitude entre les échantillons, on prélevait d'abord les n° 8 à 14 sur un plant fleuri ayant sa première main mâle découverte, puis les n° 1 à 7 sur le plus proche plant non fleuri au stade convenable ; et on recommençait sur une autre paire de plantes, jusqu'à avoir assez de matière végétale pour l'analyse.

Dessiccation à 80°C, broyage. Dosages de N par distillation, de P par le bleu de molybdène, sur minéralisat H_2SO_4/H_2O_2 . Dosages de K par photométrie de flamme, de Ca, Mg, Mn par absorption atomique, sur cendres obtenues à 550°C et dissoutes dans HCl dilué.

RÉSULTATS

Résumés dans le tableau 2, ils ont été analysés statistiquement pour chaque élément par la technique des composantes principales.

UNA COMPARACIÓN DE MÉTODOS DE MUESTREO DIAGNOSTICAL EN PLÁTANOS (BANANOS)

INTRODUCCIÓN

MARTIN-PRÉVEL (1974) subrayó la diversidad de los métodos en uso para el análisis foliar del plátano (banano) y propuso el estudio de una serie de muestreos con fin de llegar a un método internacional de referencia. En los 9 últimos años ha acumulado el Centro de Investigaciones y de Desarrollo del Winban (Asociación de los productores de plátanos de las Islas bar lo Viento) numerosos datos de análisis foliar en apoyo de la experimentación y para aconsejar a los productores.

La definición del muestreo utilizado por el Winban evolucionó por parte desde TWYFORD y COULTER (1964) de manera que la posición de las hojas sea designada según el criterio empleado por la mayoría de los otros investigadores. En este artículo, el muestreo «Estandar» representa dos fajas de 5 a 10 cm de anchura tomadas de ambos lados del limbo al centro de la hoja en posición III.

MÉTODOS

En diciembre de 1974 y enero de 1975 se tomaron de veinte plataneras, cinco en cada isla (cuadro 1), la muestra «Estandar» y otras 13 muestras, por mayor parte según las sugerencias de MARTIN-PRÉVEL (1974) : ver fig. 1. La fase sin flor es posterior a la iniciación floral. La «mas vieja de las hojas vivas» tiene más de los 50 % de su superficie aún verde.

Para asegurar la mejor similitud entre las muestras, se tomaban en primer lugar los n°s 8 a 14 de un plantón con racimo teniendo su 1ª mano masculina descubierta, luego los n°s 1 a 7 del plantón sin flor en la fase conveniente lo mas próximo al precedente ; entonces se hacía lo mismo de un otro par de plantones, hasta que la materia vegetal sea suficiente para el análisis.

Desección a 80°C, trituración. Determinaciones de N por destilación, de P por el azul de molibdeno, en un mineralizado H_2SO_4/H_2O_2 . Determinaciones de K por fotometría de llama, de Ca, Mg, Mn por absorción atómica, en cenizas obtenidas a 550°C y disueltas en HCl diluto.

RESULTADOS

El cuadro 2 presenta un resumen de los resultados, que fueron analizados para cada elemento por la técnica de las componentes principales.

L'effet du niveau global de l'élément dans la plante (1ère composante principale) est toujours le principal responsable de la variation entre les observations, ce qui signifie un haut degré d'accord, dans l'ensemble, entre les divers modes de prélèvement (tableau 3).

L'analyse à l'aide de la matrice de corrélation, incluant à la fois la variation intra- et inter-îles, est préférable à l'analyse utilisant la matrice de covariance : celle-ci explique une plus grande part de la variance totale par la 1ère composante principale, mais accorde trop d'importance aux modes d'échantillonnage dotés d'une variabilité excessive. On a également utilisé la matrice de corrélation de la variation intra-îles réunies, après élimination de la variation inter-îles. Le degré d'accord général entre échantillonnages est apprécié dans la colonne centrale du tableau 3.

Les valeurs et signes des coefficients de la 2e composante principale révèlent, dans chaque série de 7 modes d'échantillonnage, un désaccord entre un ou deux d'entre eux d'une part, un groupe de deux autres d'autre part, expliquant une bonne part de la variation restante (3 dernières colonnes du tableau 3). Ainsi, 6 et 7 sont en contraste avec 4 et 5 pour N, P et S ; cas analogue pour Ca, Mg et Mn ; pour K il y a contraste avec 3 et 5. De même 11 et 14 contrastent pour tous les éléments avec 10 et/ou 13. Tous ces contrastes semblent physiologiquement acceptables.

Par ailleurs, avec les deux matrices de corrélation, le niveau général de l'élément explique une plus grande part de la variation pour N, P, S chez les non-fleuris, pour K, Ca, Mg, Mn chez les fleuris : résultat inverse pour le contraste entre méthodes.

DISCUSSION

L'enquête a été axée sur le degré d'accord entre «l'histoire racontée» par les divers échantillons, à propos de situations aussi différentes que possible.

Pour ne pas accorder de préférence a priori à l'une des méthodes, on a réparti les plantations en trois classes d'effectif sensiblement égal, d'après la moyenne des quatorze échantillons : niveau général faible, moyen, élevé en chacun des éléments (rectangles noirs sur les figures). Puis on a comparé la discrimination entre les trois classes de chaque méthode avec celle de la moyenne. L'étroit accord entre les conclusions de cet examen et celles de l'analyse en composantes principales montre que la moyenne générale est une bonne base pour la ségrégation des plantations.

Ces classes n'ont pas de valeur diagnosticielle, car pour avoir assez de bananiers à prélever on a dû s'adresser uniquement à des plantations déjà convenablement fertilisées. La distribution normale explique que la classe moyenne ait l'amplitude de variation la plus faible (sauf pour Mn) ; il

El efecto del nivel global del elemento en la planta (1a. componente principal) tiene siempre la mayor parte de responsabilidad de la variación entre las observaciones. Esto significa un alto grado de acuerdo, para la generalidad, entre los distintos modos de muestreo (v. cuadro 3).

El análisis con ayuda de la matriz de correlación, que incluye ambas variaciones inter- y intra-islas, es preferible al análisis utilizando la matriz de covariancia : aunque esta explique una mayor parte de la variancia total por la primera componente principal, atribue una importancia demasiado a los modos de muestreo poseyendo una variabilidad excesiva. Se utilizó también la matriz de correlación de la variación intra-islas reunidas, después de la eliminación de la variación inter-islas. En la columna central del cuadro 3 se puede apreciar el grado de acuerdo general entre muestreos.

Las valores y los signos de los coeficientes de la 2a componente principal revelan un desacuerdo, en cada serie de 7 modos de muestreo, entre uno o dos de ellos por una parte, un grupo de dos otros por otra parte. Este explica una gran parte de la variación restante (3 últimas columnas del cuadro 3). Así 6 y 7 contrastan con 4 y 5 para N, P y S ; el caso es similar para Ca, Mg y Mn ; para K hay contraste con 3 y 5. Igualmente 11 y 14 contrastan para todos los elementos con 10 y/o 13. Todos estos contrastes parecen fisiológicamente aceptables.

Por otra parte, con ambas matrices de correlación, el nivel general del elemento explica una mayor parte de la variación para N, P, S en las plantas sin flor, para K, Ca, Mg, Mn en las plantas con flor, y al contrario en cuanto al contraste entre métodos.

DISCUSIÓN

La encuesta fué orientada en el grado de acuerdo entre «la historia contada» por las distintas muestras concernientes a situaciones lo más diferentes que posibles.

A fin que no se atribuyese ninguna preferencia inmotivada a cualquier método, se repartieron las plantaciones en 3 clases de sensiblemente el mismo número de fincas, según el promedio de las 14 muestras : nivel general bajo, medio, elevado para cada elemento (rectángulos negros en las figuras). Luego se comparó la discriminación de cada método entre las 3 clases con la del promedio. Del estrecho acuerdo entre las conclusiones de este examen y aquellas del análisis en componentes principales se deduce que el promedio general es una buena base para la segregación de las fincas.

Estas clases no tienen un valor diagnostical, porque se debían tomar muestras únicamente de fincas ya bastante bien abonadas, para tener un número suficiente de planto-

en résulte un certain degré de confusion avec les deux autres classes, correspondant aux grisés sur les figures.

Azote.

Son diagnostic est de première importance, d'autant plus que l'analyse du sol est de peu de secours pour cet élément.

Le limbe extérieur est toujours plus riche que l'intérieur, même si le niveau général diminue en fonction des lieux ou de l'âge de la feuille ou de celui de la plante (effet léger dans ce dernier cas, au stade précoce de développement du régime qui a été utilisé). La teneur des organes conducteurs (pétiotes et nervures), très basse, est très peu affectée par l'âge de la feuille ou de la plante : elle refléterait plutôt les disponibilités du moment que le niveau de la plante.

Les teneurs élevées étant considérées par beaucoup comme un facteur important du choix méthodologique (cf. adoption de la feuille III), il est regrettable que, dans certains pays, on doive éliminer le limbe externe en raison de la fréquence des brûlures marginales. Celles-ci sont rares aux Iles du Vent, où l'échantillon «Standard» donne des résultats fiables et permet ici la meilleure discrimination entre classes faible et élevée (figure 2).

La contraction du graphique, par disparition des extrêmes, quand on passe à la feuille I montre la meilleure valeur diagnosticielle de la feuille III : la feuille I apparaît nourrie en priorité en cas de déficit azoté, tandis que les excédents s'accumulent plutôt dans la feuille III.

La figure 2 indique également que l'échantillon Winban standard et le limbe extérieur sont les meilleurs indicateurs en cas d'excès, donc de gaspillage coûteux en engrais azotés.

Phosphore.

Malgré les différences de niveau et d'étendue du domaine de variation selon les échantillons, on ne peut guère tirer de conclusions sur leurs valeurs diagnosticielles, aucun cas ne s'approchant des niveaux reconnus comme correspondant à un besoin net d'apports phosphoriques.

Soufre.

Sa déficience, bien connue aux Iles du Vent (1967), est corrigée par l'emploi constant d'engrais contenant en moyenne 4 p. 100 de cet élément : on est donc dans le même cas que pour P. Toutefois la pétiole et la nervure de la plus vieille feuille vivante paraissent intéressants. La baisse des teneurs après floraison, surtout dans les nervures (où les classes faible et forte se distinguent alors très mal), peut indiquer une fourniture insuffisante de S à ce stade, susceptible d'affecter la qualité du fruit.

nes que muestrear. La distribución normal explica que la clase media tenga la mas débil amplitud de variación (salvo para Mn) ; de esto resulta un cierto grado de confusión con las otras dos clases, que se representa por zonas sombreadas en las figuras.

Nitrógeno.

Su diagnóstico tiene una importancia tanto más extrema que el análisis del suelo es de poca ayuda para este elemento.

El limbo exterior presenta siempre una mayor riqueza que el interior, aún si el nivel general disminuye en función de los lugares o de la edad de la hoja o de la edad de la planta (efecto ligero en este último caso, en la fase juvenil de desarrollo del racimo que fué utilizada). El nivel en los organos de conducción (pecíolos y nervios), que es muy bajo, es poco afectado por la edad de la hoja o de la planta : él reflejaría más bien las disponibilidades del momento que el nivel en la planta.

Puesto que los niveles elevados son considerados por muchos como un factor importante para la elección metodológica (v. selección de la hoja III), se puede deplorar que en algunos países se deba eliminar el limbo externo por causa de la frecuencia de quemaduras marginales. Estas son raras en las Islas bar lo Viento, donde la muestra «Estandar» da resultados fiables y permite aquí la mejor discriminación entre las clases baja y elevada (fig. 2).

El estrechamiento del gráfico, por desaparición de los extremos, cuando se trata de la hoja I, demuestra el mejor valor diagnostical de la hoja III : parece que la hoja I es alimentada con prioridad en caso de falta de nitrógeno, mientras los excedentes eventuales se acumulan preferiblemente en la hoja III.

Igualmente indica la fig. 2 que la muestra Winban estandar y el limbo exterior son los mejores indicadores en case de exceso, entonces de derroche costoso de abono nitrogenado.

Fósforo.

A pesar de las diferencias de nivel y de extensión del campo de variación según las muestras, no se puede sacar casi ninguna conclusión sobre su valor diagnostical, pues ningún caso alcanzaba los niveles reconocidos correspondientes a una necesidad clara de suministro fosfórico.

Azufre.

Su deficiencia, bien conocida en las Islas bar lo Viento (1967), se corrige por el empleo constante de abonos con un contenido medio de 4 % de este elemento : así el caso está el mismo que para P. Sin embargo el pecíolo y el nervio de la mas vieja de las hojas vivas parecen interesantes.

Potassium.

C'est le seul élément plus concentré dans les zones internes du limbe que dans les zones externes.

Ses niveaux extrêmement diversifiés dans le pétiole et la nervure sont à rapprocher de sa mobilité et de son incapacité à former des combinaisons organiques stables. Mais, malgré les apparences de la figure 2, leur relation avec l'état général de nutrition potassique de la plante apparaît peu étayée, les plantes indubitablement bien fournies présentant des niveaux très faibles (jusqu'à 0,5 p. 100) aussi bien que très élevés. Ces organes sont probablement trop influencés par les conditions immédiates ou la redistribution dans la plante. Des niveaux critiques y sont difficilement imaginables.

En revanche les limbes sont en bonne relation avec les faits observés sur la plante, le sol et les engrais, conformément à l'expérience du Winban avec son échantillon standard. Certes, dans celui-ci, l'écart n'est pas très grand entre le niveau de déficience (2,2 p. 100) et le niveau très satisfaisant de 3,2 p. 100 correspondant à la plus faible valeur dans la présente enquête : mais on a obtenu un C.V. de 5 à 7 p. 100 dans les limbes contre 60 à 70 p. 100 dans les pétioles et nervures.

Calcium.

L'accord entre tous les échantillons est bon, notamment entre le Winban standard et les pétiole et nervure de la plus vieille feuille. La distinction entre faible et élevé est meilleure dans le limbe interne que dans l'externe.

Le niveau critique de Ca est peu établi aux Iles du Vent, où on ne connaît pas de cas de déficience caractérisée. On peut, notamment par l'emploi judicieux des engrais potassiques, obtenir des rendements excellents sans baisse de qualité avec des teneurs dans l'échantillon standard inférieures au niveau critique provisoire, qui a pourtant déjà été abaissé de 0,5 à 0,45 p. 100. Le calcium compte donc peu, actuellement, dans le choix de l'échantillon.

Magnésium.

L'échantillon «standard» donne satisfaction ici comme dans son usage passé aux Iles du Vent, et on n'entrevoit aucune amélioration par l'emploi d'un autre tissu, bien que le pétiole ou la nervure d'une feuille âgée puissent se montrer aussi utiles. D'après l'analyse en composantes principales, toutes les méthodes se valent sensiblement.

Manganèse.

La première composante principale explique presque 100 p. 100 de la variation, signifiant une valeur identique de tous les échantillons. Cela vient en partie d'une gamme de conditions de nutrition (de la presque déficience à la

La diminution de los niveles después de la floración, sobre todo en los nervios (donde las clases baja y elevada se distinguen muy poco en este momento), puede indicar un suministro insuficiente de azufre en esta fase con riesgo de disminuir la calidad del fruto.

Potasio.

El único elemento más concentrado en las zonas internas del limbo que en las zonas externas.

Sus niveles extremadamente diversificados en el pecíolo y en el limbo pueden compararse con su movilidad y su incapacidad a formar compuestos orgánicos estables. Pero, a pesar de lo que parece en la fig. 2, su relación con el estado general de nutrición potásica de la planta se muestra poco apuntalada, puesto que plantas indudablemente bien suministradas presentan niveles lo mismo muy bajos (hasta 0,5 %) que muy altos. Estos órganos son probablemente demasiado influidos por las condiciones del momento o por la redistribución en la planta. Niveles críticos están difícilmente imaginables en ellos.

En cambio se encuentra en los limbos una buena relación con las observaciones sobre la planta, el suelo y los abonos, en conformidad con la experiencia del Winban con su muestra estandar. Por cierto, en esta última, no hay una diferencia muy grande entre el nivel de deficiencia (2,2 %) y el nivel muy satisfactorio de 3,2 % correspondiente al menor valor de esta encuesta ; pero C.V. de 5 a 7 % fueron obtenidos en los limbos en comparación con 60 a 70 % en los pecíolos y nervios.

Calcio.

Hay un buen acuerdo entre las distintas muestras, especialmente entre el Winban estandar y los pecíolos y nervios de la hoja viva más vieja. Bajos y elevados se distinguen mejor en el limbo interno que en el limbo externo.

El nivel crítico del Ca no está bien establecido en las Islas bar lo Viento, donde no se conocen casos de deficiencia definida. Por el empleo juicioso de los abonos potásicos en particular, es posible obtener rendimientos excelentes sin disminución de calidad, con niveles en la muestra estandar inferiores al nivel crítico provisional, que fué ya sin embargo bajado de 0,5 a 45 %. Luego, el calcio cuenta presentemente por poco en la elección de la muestra.

Magnesio.

La muestra «estandar» es satisfactoria aquí como en su uso pasado en las Islas bar lo Viento, y no se ve ningún mejoramiento posible por el empleo d'otro tejido ; sin embargo el pecíolo o el nervio de una hoja vieja podría mostrarse de la misma utilidad. Según el análisis en componentes principales, todos los métodos tienen sensiblemente el mismo valor.

presque toxicité) beaucoup plus large que pour tous les autres éléments.

CONCLUSIONS

Cette étude confirme la valeur diagnosticielle de l'échantillon Winban standard, notamment pour N et K, fertilisants les plus nécessaires aux Iles du Vent.

Pour Mg et Mn, tous les échantillons se valent.

Pour P et S, l'absence de valeurs faibles rend le choix difficile. La possibilité d'une amélioration par rapport à l'échantillon «standard», habituellement satisfaisant, peut cependant exister, mais les indications sont en faveur du limbe extérieur de la feuille I pour P, du pétiole et de la nervure de la plus vieille feuille pour S (avec, comme pour N, une supériorité du stade non fleuri).

Ca constitue une catégorie à part. Difficile à apprécier faute de niveau critique bien établi, il paraît bien discriminé par tous les échantillons, sauf les limbes externes avant floraison.

Aucun échantillon unique ne s'est donc montré supérieur à la partie centrale du limbe de la feuille III, largement utilisée.

Manganese.

La primera componente principal explica casi los 100 % de la variación, lo que significa un valor idéntico para todas las muestras. Esto viene en parte de una diversidad de condiciones de nutrición (de una casi-deficiencia a una casi-toxicidad) mucho más amplia que para todos los demás elementos.

CONCLUSIONES

Este estudio confirma el valor diagnóstico de la muestra Winban «estandar», en particular para los fertilizantes más necesarios en las Islas bar lo Viento : N y K.

Para Mg y Mn, todas las muestras tienen el mismo valor.

Para P y S, la ausencia de valores bajas hace la elección difícil. Sin embargo, la posibilidad de mejoramientos respecto a la muestra «estandar» que habitualmente satisface, puede existir, pero las indicaciones favorecen el limbo externo de la hoja I para P, el pecíolo y el nervio de la hoja más vieja para S (con una superioridad de la fase sin flor, así como para N).

El Ca constituye una categoría especial. Mientras la falta de nivel crítico bien establecido hace su apreciación difícil, él parece bien discriminado por todas las muestras con excepción de los limbos externos antes de la floración.

Entonces ninguna muestra sola apareció superior a la parte central del limbo de la hoja III, ampliamente utilizada.

