#### Resistance

# Relationship of Rust Severity and Plant Nutrients in Sugarcane

D. L. Anderson and J. L. Dean

First author, University of Florida, Everglades Research and Education Center, Belle Glade 33430; and second author, U.S. Department of Agriculture, Agricultural Research Service, Canal Point, FL 33438.

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#### ABSTRACT

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The objectives of the study were to determine if the intensity of rust development on 12 sugarcane clones was associated with the nutrient status of infected leaf tissues and to determine appropriate methods for interpretation of rust and tissue analysis data. The Diagnosis and Recommendation Integrated System, in conjunction with nutrient concentration levels, was useful in identifying nutrients likely to be important in the rust-nutrient relationship in sugarcane. Regression

analyses of tissue data and a variance-ratio approach both indicated that N, P, Cu, Mn, and Zn influenced rust intensity. However, only the variance-ratio approach defined nutrient imbalances and high or low nutrient concentrations within infected leaves that were associated with rust disease intensity. The results imply that in some circumstances, certain nutritional or edaphic conditions favor rust development on sugarcane and that control of rust may be possible by modifying the nutritional status of the plant.

Additional key words: disease resistance, Puccinia melanocephala, Saccharum spp., variance-ratio.

Rust caused in sugarcane (Saccharum spp.) hybrids by Puccinia melanocephala H. Syd. and P. Syd. has posed a serious threat to sugarcane production in Florida and many other areas in recent years (20). New races of P. melanocephala have been reported in Florida (7), and a number of sugarcane clones previously considered resistant to rust in the early stages of testing have shown susceptibility in later stages. This has occurred repeatedly in the past 2 yr in the Florida sugarcane breeding programs. Since these clones had previously remained rust free for several years in yield trials where all replications of susceptible clones were heavily rusted, and in some cases at several locations, escape in early stages of testing may be ruled out and the rise of new races of the rust pathogen is the only plausible explanation (7). Sugarcane has long breeding and crop cycles (i.e., several ration crops), and it is not clear whether breeding for resistance will be adequate to control rust if new races continue to develop. This issue has stimulated an interest in exploring supplementary methods to control rust. Management of soil fertility is one possibility.

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Sugarcane rust in Florida has been severe on sugarcane grown on organic soils formerly used for vegetable production that are high in soil fertility and poorly drained. It has also been observed that rust on highly organic soils is often less severe or nonexistent near the edges of fields close to limestone roads and ditch spoil banks. From these observations, it is hypothesized that rust development on sugarcane may be affected by the soil and plant nutrient status. Information pertaining to sugarcane rust and nutrition was lacking; however, a number of studies of other plant hosts and rust pathogens indicated that application of certain major nutrients to the soil either increased or reduced rust development (Table 1). The lack of agreement in the literature suggested that variability in soil fertility levels may result in greater likelihood of rust and that specific levels of available plant nutrients may reduce rust intensity. The lack of agreement in the literature also raises questions regarding data interpretation and appropriate statistical approaches.

The objectives of this study were to determine if the intensity of rust development on sugarcane is associated with the nutrient status of infected leaf tissues and to find statistical methods appropriate for interpretation of rust and tissue data. Attention was directed to nutrient concentrations present in leaf tissues of different maturities from sugarcane clones of different disease resistance. The Diagnosis and Recommendation Integrated System (DRIS) (2-4,17) was investigated as a possible approach for characterizing and relating the plant nutrient status to rust intensity.

## MATERIALS AND METHODS

In June of 1982, while rust severity was high, tissue samples from cultivar CP63-588 growing in commercial fields on organic soils in the Everglades Agricultural Area of Florida were analyzed (9) for N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu (Table 2). The topmost leaf showing a visible dewlap (TVD) was taken from 15-20 plants each with and without rust. Leaves were dried 3 days at 60 C and then ground in a Wiley mill to pass a 0.85-mm screen (20-mesh) prior to analysis.

On 7 June 1983, rust intensity ratings were made on 12 sugarcane clones planted 8 November 1982 in a randomized complete block design with five replications, on an unfertilized Lauderhill muck (Euic, hyperthermic Lithic Medisaprist) at the Everglades Research and Education Center, Belle Glade, FL. Each plot was 1.22 m long by 1.52 m wide. Whole-plant rust intensity ratings were made on a 0-9 scale (14), with 0 indicating no sporulating pustules and 9 indicating densely packed, large, and heavily sporulating pustules on the underside of the leaf blade. The clones were selected to represent the full range of response to rust development, i.e., from very resistant to very susceptible (Table 3). Clones with "low"

TABLE 1. Literature citing the influence of soil fertility on rust development<sup>y</sup>

Plant						
host	Rust pathogen	N	P	K	General	Ref
Barley	Puccinia hordei	+	+-	+		1
	Puccinia hordei	+				31
Coffee	Hemileia vastatrix	-	-+	+		10
	Hemileia vastatrix	0	0	0		18
Cowpea	Uromyces phaseoli	+-	0	0		21
Pine	Cronarium fusiforme				+	22
	Cronarium fusiforme	+	+	0		23
	Cronarium fusiforme				+	24
Poplar	Melampsora larici-populina	+	+	-		28
3.0 30. <b>4</b> 3.000.00	Melampsora larici-populina			_		29
Ryegrass	Puccinia coronata	+				13
	Puccinia coronata	+				15
Wheat	Puccinia graminis	+				5
	Puccinia recondita					
	Puccinia striiformis					
	Puccinia graminis	+	_	_		6
	Puccinia recondita					
	Puccinia graminis	+				12
	Puccinia graminis	+				16
	Puccinia hordei	-				19
	Puccinia striiformis	+		-		25
	Puccinia graminis	+	-			27
	Puccinia recondita					
	Puccinia graminis	+0	+0	+(	)	32

<sup>&</sup>lt;sup>y</sup>This table is a generalization of reported findings involving rust disease on the host species and its relationship to soil fertility.

disease intensity ratings (0-3) indicated some clonal resistance to rust. Clones with ratings greater than 3 indicated a "high" susceptibility to rust.

After disease intensity ratings were assigned, four leaves were sampled from each of 15–20 plants from each plot and designated 0, 1, 2, and 3 in ascending order of maturity. Sample 0 consisted of three or four immature leaves from each plant with the dewlap still hidden in the whorl (8). Samples 1, 2, and 3 consisted of the blade tissues from leaves showing the first, second, and third visible dewlaps (VD), respectively, from the top of the plant down. The samples were dried, ground, and digested (33) prior to analyses for total N and P by using spectrophotometric procedures (30) and for total K, Ca, Mg, Fe, Mn, Zn, and Cu by using atomic absorption spectrophotometry.

Florida sugarcane DRIS reference norms were established from tissue data by using procedures developed by Beaufils (1) and Elwali and Gascho (9) and the program adapted for the Apple III computer. Simple regression correlation (r), stepwise partial regression  $(R^2)$ , means, and variances were performed by using SAS (26). The variance-ratio approach (11) was used to determine nutrient concentration, nutrient concentration ratio, or DRIS index differences between plants rated either "high" or "low" in disease severity. Significant differences between the "low" and "high" rust susceptible clones for the various nutrient conditions were accomplished by determining the ratios of the nutrient concentration, or DRIS index variance at "high" rust incidence to the variance at "low" rust incidence ( $V_{\text{high}^-}/V_{\text{how}^-}$ ). The significant variance-ratio at the 1% level (11) is calculated by:

$$1\%$$
 significance level =  $e^{2z}$  (1)

in which

$$z = [2.3263/(h-1.4)] - 1.235[(1/n2) - (1/n1)],$$
 (2)

$$h = 2/[(1/n_2) + (1/n_1)], (3)$$

 $n_1$  and  $n_2$  are the degrees of freedom, and  $n_1$  must always correspond with the greater mean square. Significant differences between the "low" and "high" rust susceptible clones indicated that leaf nutrient concentrations were significantly different between the two populations and that leaf nutrient concentrations were correlated with rust severity.

### RESULTS

In the initial study, fields were chosen in which rust on sugarcane cultivar CP63-588 was less severe along borders of fields affected by limestone spoils and higher mineral content. Commercial fields showing differences in rust intensity within each field (Table 2) failed to indicate a correlation of single nutrients with rust severity. In contrast, by using all 12 cultivars at a single location, simple regression between single tissue nutrients or the nutrient DRIS indices (indicated in text or table by "I") and rust ratings indicated specific trends associating nutrient content with rust severity (Table 4). Simple regression indicated that DRIS indices were useful in the

TABLE 2. Nutrient concentrations in the dry matter of topmost visible dewlap leaves of CP63-588 with and without rust disease

		Nutrient levels								
		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Field	Tissue sampled	$g \cdot g^{-1} \times 100$					μg·g <sup>-1</sup>			
1	Rusted	1.98	0.23	1.66	0.27	0.18	60	133	22	1.8
	Nonrusted	2.07	0.20	1.64	0.24	0.15	58	37	15	0.9
2	Rusted	2.38	0.24	1.78	0.29	0.17	67	122	17	2.2
	Nonrusted	2.02	0.20	1.62	0.32	0.16	64	48	16	2.2
3	Rusted	2.07	0.24	1.91	0.31	0.17	69	73	25	2.9
	Nonrusted	2.25	0.21	2.09	0.38	0.11	70	26	23	3.8
4	Rusted	2.22	0.22	1.78	0.32	0.11	65	32	20	3.2
	Nonrusted	2.21	0.23	1.83	0.31	0.15	65	79	24	2.2

Symbols: + indicates that an increase of nutrient increased rust disease, 0 indicates no effect, - indicates that an increase of nutrient decreased rust disease, and +- or +0 indicates that both responses were observed in the study.

analyses. Tissue concentrations of Zn and P in leaf samples 0 and 1 were significantly related to the level of rust development. The concentration of N varied with leaf maturity and was positively correlated with rust development in three of the four leaf tissues. Based on simple regression analysis, plants with less N in young leaf tissues than older mature leaves showed less rust. Plants high in Ca in the third VD leaf had lower rust incidence than those of lower Ca content.

DRIS indices or nutrient tissue concentrations by themselves were more poorly correlated ( $R^2$ ) to rust intensity than when used together to develop the "best-fit" regression model. Although partial regression analysis using the DRIS index and linear and second-order polynomial nutrient concentration terms as independent variables indicated that specific combinations of plant

nutrients may be associated with the level of rust development (Table 4), simple regression accounted for most of the model variability. The highest correlations were attained by using nutrient concentration data from the 0 and third VD leaves. The N and Ca contents were consistently associated with the level of rust development in all leaf tissues. Phosphorus, Zn, and Fe were associated with the level of rust to a lesser degree.

Results obtained by using the variance-ratio approach (11) showed that P, Fe, Mn, and Zn were consistently involved and associated with rust intensity in all leaf tissues (Table 5). Nitrogen was significantly (P > 0.01) associated with rust intensity in all but the first VD leaves. Unlike regression, the variance-ratio analyses indicated that nutrient ratios were important but also deemed the importance of many of the same nutrients indicated by regression.

TABLE 3. Mean nutrient contents by cultivar and by leaf sample (n = 240)

	Mean nutrient contents									
Cultivars	N	P	K	Ca	Mg	Mn	Cu	Zn	Fe	Maa.a
Leaf samples	g·g <sup>-1</sup> × 100					μg·g <sup>-1</sup>			Mean rust rating <sup>z</sup>	
			Acros	s all leaf sa	imples (n =	= 20) for cu	ıltivars			
CP70-1133	1.95	0.19	1.33	0.41	0.21	14.7	4.0	13.7	75.2	0.0 "low"
CP68-1026	1.90	0.19	1.39	0.55	0.23	16.4	3.7	15.6	67.9	0.6 "low"
US72-1210	1.74	0.18	1.02	0.49	0.27	21.3	5.0	16.6	74.9	2.8 "low"
CP71-1086	1.77	0.20	1.33	0.45	0.23	16.8	4.7	14.7	62.6	3.0 "low"
CP68-1067	1.84	0.21	1.40	0.48	0.22	15.9	4.6	18.1	72.6	4.0 "high"
CP65-357	1.84	0.21	1.35	0.51	0.20	21.1	1.0	19.7	92.0	4.8 "high"
CP63-588	2.00	0.19	1.17	0.53	2.26	11.8	3.9	16.8	73.8	5.0 "high"
CL41-223	1.78	0.17	1.56	0.44	0.17	13.5	3.8	15.5	70.0	6.8 "high"
CP78-2042	1.89	0.22	1.37	0.41	0.24	16.0	3.9	19.2	72.4	7.0 "high"
H49-5	1.81	0.22	1.35	0.41	0.21	14.7	4.0	19.8	79.1	9.0 "high"
B43-62	1.77	0.19	1.36	0.42	0.24	12.9	3.9	16.2	71.7	9.0 "high"
CP78-1735	1.78	0.19	1.22	0.40	0.20	17.0	3.6	15.8	77.4	9.0 "high"
			Acros	s all cultiva	ars (n = 60)	) for leaf sa	imples			
0	1.74	0.20	1.36	0.34	0.21	17.4	4.0	19.4	68.4	
1	1.93	0.21	1.35	0.42	0.23	15.6	4.0	16.7	77.8	
2	1.88	0.20	1.30	0.49	0.22	15.9	4.1	15.6	72.6	
3	1.78	0.19	1.30	0.57	0.23	16.0	4.2	15.7	77.6	

Ratings are an average of five replications. Zero indicates no spore production could be detected, "low" indicates a resistance to rust disease, and "high" indicates high rust disease susceptibility.

TABLE 4. Rank of importance determined by simple correlation (r) and multiple (partial) correlation  $(R^2)$  between specific plant nutrients or nutrient DRIS indices and rust ratings on leaf tissues of different maturities  $(n = 57)^y$ 

Leaf sampl	e Ranking by simple regression correlation (r)	Ranking by partial regression correlation $(R^2)^r$	
0	Zn (0.45**) > MgI (-0.32*) > P (0.30*) > ZnI (0.28*) > N (0.28*)	$+N^2 > -FeI > -NI > +Fe > +ZnI_2 > -Ca > -Cu^2 > -Zn^2$	0.49**
1	PI(0.32*) > MnI(-0.31*) > P(0.31*) > ZnI(0.31*) > Cu(0.27*)	-MnI > +Zn > -CaI > +PI > -Cu > -N	0.34**
	N (-0.30*)	$-N > -Cal > +Ca > -Mnl > +Fel > +Fe > -Ca^2 > +P^2$	0.33**
3	Ca $(-0.51**)$ > N $(-0.47**)$ > CaI $(-0.37**)$	$-N > +Ca^2 > +N^2 > -K^2 > +KI > -Ca > +PI > -CaI$	0.55**

YUsing all 12 cultivars; + or - indicates the sign of the coefficient term (B value) within each model; squares (e.g.,  $N^2$ ) of single nutrient terms are second-order model terms; DRIS indices are indicated by I (e.g., FeI); \*\* indicates significance at P = 0.01 and \* indicates significance at P = 0.05.

Partial regression correlation coefficient of model having the largest  $R^2$  and highest significant F statistic determined the "best-fit" model.

TABLE 5. Significant (P>0.01) nutrient concentrations, concentration ratios, and DRIS index ranges that characterize leaf tissues "low" in rust intensity as determined by the variance-ratio approach<sup>z</sup>

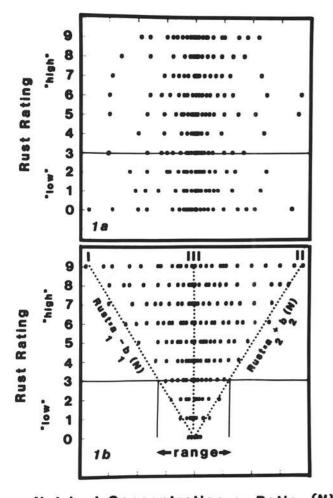
Leaf sample							
0	1	2	3				
Fe/Mg 200-460 K/Mg 2.5-12.5 N/P 7.0-10.5 Zn/Mn 0.5-2.0	$\begin{array}{cccc} Zn/Mn & 0.5-1.8 \\ Zn/Fe & 0.10-0.35 \\ Fe/Mn & 3-10 \\ MnI & (-18)-1 \\ Zn & 10-24 \\ P & 0.17-0.2 \end{array}$	MnI (-12)-0 Fe/Mn 3-11 Zn/Mn 0.5-1.5 Mn/Mg 30-140 Mn/N 4-14 Fe/P 260-520 Mn 7-24	Fe/Cu 8-80 FeI (-26)-65 P/Cu 0.015-0.150 Zn/Cu 1.5-12 Mn/Cu 1-14 N/Cu 0.1-1.3 K/Cu 0.1-1.1 NI (-25)-15 Fe/K 40-90				

<sup>&</sup>lt;sup>z</sup> Units of concentration used: N, P, K, and Mg in g · g <sup>-1</sup> × 100 and Fe, Mn, Zn, and Cu in  $\mu$ g · g <sup>-1</sup>. Values outside of these ranges are characteristic of cultivars "high" in rust intensity and more susceptible to rust development. DRIS indices are indicated by the symbol "I" (i.e., Fel, Mnl, Nl).

### DISCUSSION

The nutrient level approach discussed by Elwali and Gascho (9) commonly has been used to develop reference values for plant nutrient status and can be used to interpret plant nutrient concentration effects on yield and growth. A major problem with this approach is that nutrient concentration is variable and depends upon the plant part sampled, clone, growth rate at the time of sampling, developmental stage, soil fertility or type, and environmental conditions. This variability in nutrient content and lack of apparent correlation of nutrient content with rust development or severity were demonstrated by the nutrient status of the TVD leaf tissues from different commercial fields of sugarcane cultivar CP63-588 with and without rust (Table 2). These results were representative of our observations from other commercial field data. Although it was readily apparent that rust is influenced by soil or nutrient conditions in the field, field data did not reveal a simple relationship between rust and nutrient status of infected leaf tissue.

The different conclusions reached by researchers concerning the effects of nutrients on rust development (Table 1) indicated that both nutrient deficiencies and excesses may play a large role in disease severity. If this is the case in sugarcane, simple regression will not be adequate, since it only describes a linear relationship (Table 4). Partial regression, although no improvement on the simple regression analyses of the data (Table 4), indicated that more than one plant nutrient may be influencing the level of rust intensity. Since several significant regression relationships were noted, it indicates that more complex nutrient relationships with rust may be involved.



Nutrient Concentration or Ratio (N)

Fig. 1. Rust intensity ratings plotted against the nutrient or nutrient

Fig. 1. Rust intensity ratings plotted against the nutrient or nutrient concentration ratio (N) when: a, there is no relationship and b, the relationship can be explained by three regression models (I, II, and III).

When nutrient concentration, ratio, or DRIS index is plotted against rust intensity, one of two idealized relationships can be derived (Fig. 1a and b). Fig. 1a would indicate that no relationship between nutrient condition and rust intensity exists. Fig. 1b would be approximated by three regression equations (4) which indicate that: the particular condition causes greater rust intensity because it is in insufficient quantity and a negative regression coefficient will describe the relationship (I); the particular condition causes greater rust intensity because it is in excess and a positive regression coefficient will describe the relationship (II); and the particular condition does not affect rust intensity and the regression coefficient of zero describes the relationship (III). This method combines partial regression techniques to indicate most nutritional aspects associated with rust intensity. After significant differences in nutrient status between plants "low" and "high" in rust intensity have been determined, lines are graphically drawn along the parameters of the plotted data as depicted by Fig. 1b. The concentration "range" of single nutrients, nutrient ratios, or DRIS indices that characterize plants "low" in rust intensity was graphically determined from plotted data (Table 5). Rust intensity plotted against Fe/Mg, Zn/Mn, MnI, and Fe/Cu concentration ratios from the respective tissue samples 0 through 3 are given as examples of how these concentration "ranges" were derived (Fig. 2a-d). Concentration values outside of the "range" are associated with plants susceptible to rust because of deficient or excess nutrient conditions. As determined by this approach, the nutrients more highly associated with rust intensity than others were Mn, Zn, and Fe. Fig. 1 implies that there are conditions when there is no relationship between nutrient concentration and rust intensity, but that there may be when nutrient imbalances, deficiencies, or excesses exist. The variance-ratio approach would be adaptable to a wide range of nutritional deficiencies, adequacies, or excesses (4) that influence rust intensity.

The nutrient contents in leaves change with maturity and reflect a developmental pattern; this is particularly true in the leaf samples taken in these studies (Table 3). Some nutrients (i.e., N, P, and K) are mobile plant nutrients and are transported from one plant part to another as the nutrient demand dectates. Other nutrients may be

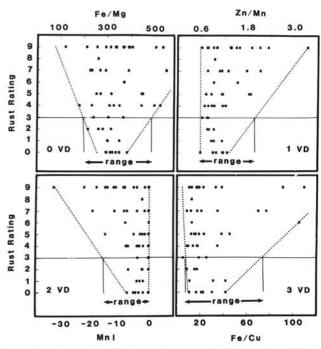


Fig. 2. Rust intensity ratings plotted against Fe/Mg, Zn/Mn, MnI, and Fe/Cu in the respective 0, 1, 2, and 3 visible dewlap (VD) leaf tissue samples. Fe/Mg, Zn/Mn, and Fe/Cu refer to the concentration ratios of the nutrients in the respective VD leaf tissues. Units of concentration used: Mg in  $g \cdot g^{-1} \times 100$  and Fe, Mn, Zn, and Cu in  $\mu g \cdot g^{-1}$ . MnI is the Mn DRIS index, where minus signs indicate deficiency of Mn in respect to the other nutrients.

immobile or only moderately mobile and thereby accumulate in older tissues. The significance of certain plant nutrients in the rust intensity-nutrient relationship is a function of leaf sample position and leaf developmental changes (Tables 4 and 5); for this reason, leaf sample positions were reported.

Two approaches to statistical interpretation of the relationship between rust intensity and the nutritional status of sugarcane were presented: the regression (simple and partial) approach and the variance-ratio approach. Simple and partial regression analyses of leaf tissue data using nutrient content and DRIS indices together indicated that N, P, and Zn were the most important nutrients associated with rust intensity. However, three failings of the regression approach used in this study are recognized: the regression models are site specific and would change under different edaphic and environmental conditions affecting nutrient availability in the soil and plant (Table 2); the regression models did not define deficient or excess plant nutrient conditions in relationship to rust severity; and the regression models did not correlate significant nutrient concentration ratios or imbalances that were defined by the variance-ratio approach. Both the regression and variance-ratio approaches indicated that similar nutrients were associated with rust intensity (i.e., N, P, Zn, Mn, and Cu). In contrast with regression, however, the variance-ratio approach reportedly is less affected by edaphic and environmental changes (4). The variance-ratio approach also interpreted nutrient concentration imbalances and defined nutrient concentration ranges under which rust intensity declined, increased, or was minimized-conditions that would otherwise require the use of several regression models.

Although it is not known whether leaf nutrient concentration conditions influence rust intensity or if rust intensity influences the leaf nutrient status, an association between rust intensity on sugarcane with the nutrient status was demonstrated. This paper does not show how to control sugarcane rust by modifying the nutritional status of the plant but points out those nutrients likely to be important in the rust-nutrient relationship. Caution should be exercised in using the cited nutrient concentration relationships and ranges (Tables 4 and 5), since statistical significance may not always be biologically significant. Consequently, efforts to control sugarcane rust by using soil amendments currently are being studied by the authors.

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