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Determination of Crop-Specific Parameters Used in Foliar Mass to Area Conversion: I. For Selected Varieties of Grapes

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Over the years several authors have developed (Gunther *et al.* 1973; Gunther *et al.* 1974; Westlake *et al.* 1973) and consolidated (Iwata *et al.* 1977) a technique for quantifying the levels of pesticide available on treated foliage. Their efforts have provided a common methodology for more consistent residue data that can be used by diverse groups to more effectively evaluate hazards that workers encounter in treated crops. The technique developed by these authors involves a standard procedure for extracting residues from the rinsate after the foliage sample has been agitated in a surfactant solution. The amount of foliar pesticide residues recovered from this analytical procedure is commonly referred to as dislodgeable foliar residues (DFR), normally expressed in units of pesticide per unit area of leaf surface. This quantity is now of increasing interest largely because of the later development of several mathematical models that can *quantitatively* relate field reentry exposure to foliar pesticide dislodgeables (Popendorf and Leffingwell 1982; Nigg *et al.* 1984; Zweig *et al.* 1984; Zweig *et al.* 1985). The practical significance of this quantitative correlation is increased by recent amendments to a state regulation that permit California growers to collect their own residue data in support of a shorter reentry interval for methomyl on grapes (California Department of Food and Agriculture 1989).

The conventional DFR procedure also involves taking leaf discs of known area as the residue samples, normally through use of a punch and die device. This sampling method has two major advantages. It provides small samples that can be readily handled in the laboratory. More importantly, since the leaf area can be determined with the area formula for a circle, it avoids the labor intensive process of measuring the leaf surface. Note that even an electronic area meter is time consuming to use because leaves may not be flat, or may overlap themselves.

Leaf punch sampling has certain drawbacks, however. The punch samplers require special maintenance and a large initial investment. The sampling regimen *per se* also depends upon the assumption, apparently not yet validated in any published article, that small leaf segments are representative of the leaf as a whole. Other related studies (Furness and Newton 1988; Sharp 1974; Speelman 1971; Stafford *et al.* 1970) suggest that the deposition of pesticide spray on leaves is far from uniform. In addition, our field experience has demonstrated that *whole leaf* samples are comparatively easier to collect.

The goal of our long-term investigation is to explore in depth the feasibility of measuring residue on whole leaves for grapes. As a first step, we undertook the task of deriving a

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mathematical formula relating leaf weight to area for grapes. The attempt of this task was to eliminate the practical problems associated with measuring leaf area.

The studies by Saiz (1990) and by others (Langer 1956; Sharratt and Baker 1986) have suggested that the area of a leaf sample can be estimated from its weight using the simple power function (also frequently referred to as the allometric equation):

$$\text{Area} = \alpha\ (\text{Weight}^{\beta}),$$

where the parameters α and β are crop-specific. For statistical computations, this power function is normally expressed as a log-transformed linear regression equation:

$$\log\ [\text{Area}] = \log\ [\alpha] + \beta\ (\log\ [\text{Weight}]).$$

Given that grape leaves vary considerably in structure and size, a question also was raised regarding the applicability of these parameters for grapes of different varieties. The immediate objective of our exploratory effort was thus twofold: 1) to estimate the parameters α_i and β_i for several selected varieties of grapes; and 2) to determine whether *a single* power function would be adequate for use to approximate leaf areas for all grapes regardless of variety.

MATERIALS AND METHODS

Nine varieties of grapes were selected for this exploratory study. These varieties, together with their sample size, are listed in Table 1. The entire data set consisted of 126 single *whole* leaf samples with each weighing between 1.0 and 5.0 grams. This weight restriction was applied in an effort to minimize the potential effect of the vein size or leaf thickness on the mass-area relationship (Frear 1935; Watson 1937). (Note that the whole leaves of some grape varieties may each weigh over 15 grams. The potential effect of their varying weight, and hence of their varying thickness as well, on the mass-area relationship will be a subject of further exploration.)

Table 1. Study samples collected in California by grape variety and type

Variety[a]	Type	County	No. of Samples
Thompson seedless	raisin	Kern	10
Dog Ridge	as root stock	Yolo	13
Couderc 1613	as root stock	Yolo	13
Ribier	table	Yolo	16
Ruby seedless	table	Sacramento	12
Flame seedless	table	Sacramento	18
French Colombard	white wine	Madera	13
Concord	red wine	Napa	9
Chenin Blanc	white wine	Napa	22
			126

[a] in sampling order.

The leaf samples were cut at the petiole where it joined the blade and collected in a plastic bag. (In practice where pesticide residue analysis is involved, the whole leaf samples should be kept in a sealed, light-weight container on ice and be weighed indirectly.) Within an hour after collection the study samples were weighed directly (without the plastic bag) on a calibrated triple beam balance (Ohaus Model N-01012-00) accurate to ± 50 mg. Their surface areas were then measured shortly thereafter with a calibrated electronic area meter (LI-COR Model LI-3100) accurate to < ± 1%.

The parameters α_i and β_i for the nine varieties, as well as for *all* varieties combined, were estimated by means of the least squares method. The 90% confidence limits for each of the estimates and other related statistics were also computed with this statistical method. In essence, a total of 10 log-transformed linear regression equations were determined. Where applicable, these linear equations were retransformed to their equivalent power functions for ease of area prediction.

A method based on the analysis of covariance (Snedecor and Cochran 1980) was used to test whether the linear regressions of leaf area on leaf mass (on log scales) were the same across varieties. The F-statistic test was used in this method to compare the regression coefficients β_i and the intercepts log $[\alpha_i]$ under the assumption that the residual variances were homogeneous. In regression analysis residual variance is a statistic which measures the variation of the differences between the actual values and the corresponding values predicted from the regression. The residual variances were compared using the Levene test for homogeneity of variance (Snedecor and Cochran 1980). One hypothesis of interest in the present study was that the regression equation based on the pooled data, herein simply referred to as the *general* regression equation, would be adequate for area prediction for all grapes under study. This would require that the parameters α_i and β_i be statistically the same across all *i* varieties.

The results of area prediction, as obtained from the *general* power function, were evaluated on the basis of 12 *test* samples. These test samples, each of which consisted of approximately 20 whole leaves, were prepared and measured by colleagues not part of this investigation team. The test samples, many of which were from varieties other than the ones studied here, were prepared under the same weight restriction as that applied to the *study* samples. However, for practical reasons the restriction was based on approximate leaf diameter (3 to 5 in.) rather than on individual leaf mass. For each test sample, the *group* area was predicted using the following modified power function:

$$\text{Group Area} = G\,[\alpha\,(\text{Group Weight}/G)^{\beta}],$$

where G is the number of whole leaves contained in the test sample. This modified function emphasizes the presumption that the parameters α and β are characteristic of a *single* leaf. (In practice the pesticide residues will be measured *collectively* from a *group* of whole leaves, rather than from a *single* whole leaf.)

RESULTS AND DISCUSSION

The individual weight and area measurements of the study samples are contained in Table 2. The parameters α_i and β_i estimated for the nine varieties and for *all* varieties combined are provided in Table 3. Other related statistics such as the correlation coefficient and the standard error of estimate are also included. The correlation coefficients were all calculated to be above 0.88, indicating an extremely high correlation between leaf weight and leaf area for the grapes under study.

All standard errors of estimate (ie, the square roots of the residual variances) were considered to be small, as evident from the high correlations noted. The largest value of the standard errors of estimate was greater than the smallest by nearly three-fold. On account of the moderately large disparity between these two extremes, some heterogeneity of the residual variances was expected. This speculation was *not*

Table 2. Weight and surface area of study samples[a]

Thompson s.		Dog Ridge		Couderc 1613		Ribier		Ruby s.		Flame s.		F. Colombard		Concord		Chenin Blanc	
weight	area	weight	area	weight	area	weight	area	weight	area	weight	area	weight	area	weight	area	weight	area
1.65	195.2	4.29	380.9	2.52	249.6	2.45	238.1	2.96	322.0	2.04	223.8	2.12	233.4	1.79	152.0	3.33	302.3
1.84	163.3	3.05	305.4	1.72	187.3	1.15	131.4	2.60	304.3	1.40	164.3	1.99	214.3	2.33	176.2	2.67	212.1
2.09	203.3	4.28	418.2	3.08	326.2	1.32	154.5	2.44	329.9	2.48	259.6	2.79	272.6	3.04	246.9	2.85	341.6
3.05	259.4	2.99	325.2	4.00	391.5	2.13	228.6	3.64	382.3	3.92	376.7	2.14	190.8	3.19	255.4	3.28	271.2
3.28	276.4	3.37	346.8	1.32	141.2	1.90	157.3	1.28	164.4	2.81	303.6	1.40	150.9	3.24	224.1	3.49	297.0
3.34	320.0	2.09	263.6	3.83	345.3	2.69	259.6	1.80	210.3	3.38	359.0	2.68	259.6	3.53	266.9	1.82	161.8
3.40	334.3	1.50	171.1	1.70	147.0	4.92	440.9	1.99	227.2	1.40	178.7	2.53	280.3	4.41	309.8	1.77	170.0
3.80	353.2	1.50	143.7	3.92	454.1	1.18	125.3	1.31	212.5	3.20	349.6	2.99	323.9	4.48	341.8	2.50	236.2
3.89	368.5	2.89	298.2	1.39	137.3	2.90	269.8	1.58	170.7	2.08	229.3	2.42	283.6	4.90	353.0	2.99	269.1
4.19	383.0	1.20	122.6	2.48	247.0	1.10	126.8	1.58	153.1	3.14	349.5	2.62	266.0			3.62	341.0
		2.28	225.3	3.90	351.9	1.90	185.1	1.98	180.1	2.06	239.1	3.08	284.5			1.68	198.3
		2.19	200.5	3.55	319.0	2.53	226.3	2.10	247.4	2.71	345.4	1.05	81.2			2.59	229.3
		4.40	352.0	3.55	293.8	2.78	312.1			1.89	207.7	1.62	146.6			3.96	317.7
						2.26	206.9			2.78	290.2					2.56	224.8
						4.22	397.6			3.94	409.3					1.81	178.5
						1.45	134.1			3.80	408.7					2.03	185.9
										1.23	152.7					3.35	271.4
										2.82	296.6					3.53	322.1
																2.31	209.2
																4.38	342.2
																3.99	304.1
																1.54	187.3

[a] weight in grams; area (both sides) in cm^2.

Table 3. Statistical results from linear regression of leaf area on leaf weight[a]

Variety	Intercept ($\log_e[\alpha]$)[b]	Slope (β)[b]	Correlation Coefficient	Stand Error of Estimate	Average $\log_e$ [Weight]	Average $\log_e$ [Area]
Thompson s.	4.692 ± 0.187	0.864 ± 0.168	0.959	0.090	1.071	5.617
Dog Ridge	4.720 ± 0.131	0.879 ± 0.128	0.966	0.106	0.940	5.547
Couderc 1613	4.634 ± 0.131	0.943 ± 0.125	0.971	0.101	0.971	5.551
Ribier	4.695 ± 0.080	0.869 ± 0.093	0.975	0.092	0.740	5.338
Ruby s.	4.859 ± 0.197	0.842 ± 0.259	0.881	0.151	0.696	5.446
Flame s.	4.841 ± 0.062	0.853 ± 0.064	0.985	0.055	0.904	5.612
F. Colombard	4.480 ± 0.146	1.162 ± 0.175	0.963	0.108	0.775	5.380
Concord	4.492 ± 0.141	0.864 ± 0.115	0.983	0.056	1.190	5.520
Chenin Blanc	4.785 ± 0.127	0.727 ± 0.122	0.917	0.102	0.992	5.506
Combined	4.752 ± 0.051	0.822 ± 0.052	0.922	0.132	0.910	5.500

[a] on natural logarithmic scales.
[b] with 90% confidence limits.

Table 4. Comparison of the log-transformed linear regression equations[a]

	df	Σx^2	Σxy	Σy^2	Regression Coefficient	Deviation from Regression df	Deviation from Regression SS	Deviation from Regression MS
Within								
Thompson s.	9	0.9895	0.8548	0.8026	0.8638	8	0.0642	0.0080
Dog Ridge	12	2.1936	1.9290	1.8189	0.8794	11	0.1227	0.0112
Couderc 1613	12	2.0942	1.9755	1.9752	0.9433	11	0.1116	0.0101
Ribier	15	3.0058	2.6106	2.3848	0.8685	14	0.1175	0.0084
Ruby s.	11	1.1235	0.9462	1.0260	0.8422	10	0.2292	0.0229
Flame s.	17	2.1982	1.8739	1.6452	0.8525	16	0.0478	0.0030
F. Colombard	12	1.2168	1.4136	1.7699	1.1618	11	0.1276	0.0116
Concord	8	0.8417	0.7271	0.6498	0.8639	7	0.0217	0.0031
Chenin Blanc	21	2.0491	1.4898	1.2891	0.7271	20	0.2060	0.0103
						108	1.0482	0.0097
Pooled, W	117	15.7124	13.8204	13.3615	0.8796	116	1.2053	0.0104
Difference between slopes						8	0.1571	0.0196
Between, B	8	2.4196	1.0885	1.0724				
W + B	125	18.1320	14.9089	14.4339	0.8222	124	2.1752	
Difference between adjusted means						8	0.9699	0.1212

Comparison of residual variances[b]: F = 0.0052/0.0034 = 1.53 (df = 8, 117); P-value > 0.10
Comparison of slopes: F = 0.0196/0.0097 = 2.02 (df = 8, 108); P-value = 0.05
Comparison of intercepts: F = 0.1212/0.0104 = 11.65 (df = 8, 116); P-value < 0.001

[a] see Snedecor and Cochran (1980), chapter 18, for computational details and for notations used.
[b] based on Levene's test for homogeneity of variance (Snedecor and Cochran 1980).

confirmed by the Levene test, however, which showed that the variance difference was statistically *in*significant (P-value > 0.10).

The results from the comparison of the regression equations, including those from the Levene test, are summarized in Table 4. As shown, the difference among the estimated slopes β_i was found to be at best marginally significant (P-value = 0.05). The difference among the estimated intercepts log $[\alpha_i]$ was calculated to be highly significant (P-value < 0.001), however. These findings suggested that only parameter α appeared to be *variety*-specific.

The results of area prediction are presented in Table 5. The *general* regression equation appeared to be adequate for leaf area prediction for all grapes. The leaf areas predicted from this equation were found to be accurate to within 15% of their actual area for 10 of the 12 test samples (ie, 83%), and to within 20% for all the test samples. The actual values were all found lying within their 90% prediction limits.

Table 5. Leaf areas of test samples predicted from the *general* regression equation[a]

Variety	Actual Area[b]	90% Lower Bound[c]	90% Upper Bound[c]	Point Estimate	% Deviation[d]
Cabernet S (N_1)[e]	3499.9	3062.7	4862.0	3858.9	10.3
Cabernet S (N_1)	4482.5	3884.6	6160.4	4891.9	9.1
Chardonnay (N_1)	4583.2	4292.4	6808.4	5406.0	18.0
Chardonnay (N_1)	5192.5	4603.8	7304.9	5799.1	11.7
Chardonnay (N_2)	4666.2	4316.3	6846.4	5436.1	16.5
Chardonnay (N_2)	5437.2	4934.5	7833.8	6217.4	14.4
Chenin Blanc (N_1)	4735.3	4241.5	6727.4	5341.7	12.8
Chenin Blanc (N_1)	5603.0	4873.1	7735.4	6139.6	9.6
Chenin Blanc (N_1)	5343.3	4824.9	7658.3	6078.7	13.8
Red Globe (K)	5682.8	4355.1	6908.3	5485.1	− 3.5
Sauvignon B (N_1)	5840.1	4679.0	7425.0	5894.2	0.9
Sauvignon B (N_1)	5339.9	4362.0	6919.2	5493.8	2.9

[a] based on Tables 2 and 3; Area = 115.8 $(\text{Weight})^{0.82}$.

[b] in cm^2 (both sides).

[c] see Snedecor and Cochran (1980), p.166, for computational details.

[d] % deviation = {(point estimate - actual area)/actual area} x 100.

[e] field location; sites N_1 and N_2 were in Napa Valley (California) whereas site K, in northern Kern County (California).

On the basis of the limited data at hand, the important question as to whether the conversion parameter β for grapes is *variety*-specific remains unresolved. This is because the nine varieties as selected might not be sufficient to serve as a representative group, since literally over 100 varieties of grapes are commonly available.

Despite the problem of data sparseness, certain observations are worth repeating. First, the correlation coefficients for all 10 regression equations were calculated to be above 0.88, indicating an extremely high degree of closeness of linear relationship between leaf mass and leaf area (on log scales) for the grapes under study. This high degree of correlation assured that the data fit well on the linear curve. Second, 83% of the leaf areas predicted from the *general* regression equation were found accurate to within 15% of their actual surface area, although this observation was based on only 12 test samples. This finding suggested that although the intercepts log $[\alpha_i]$ were found significantly different among varieties, their *actual effect* on area prediction appeared to be *negligible* or *inconsequential*. Third, the actual surface areas of the test samples were all found lying within their 90% prediction limits.

In the present study the prediction limits were set at the 90% (vs 95% or higher) confidence level. This *lower* confidence level was used to narrow the upper and lower bounds so as to ensure a tenable prediction range. For studies of this type, the sources of sampling, environmental, and biological variability contributing to the result of a significant difference in the parameters compared were bound to be enormous. In addition, in comparing *several* regression lines the probability of a difference being due to *chance* alone increases with increasing level of significance (ie, with a higher P-value). It was for these reasons that here the *comparison* tests (as those presented in Table 4) were all performed at the 1% (instead of at the conventional 5%) level of significance.

The above observations suggested that at least for whole leaf samples with a mass ranging from 1.0 to 5.0 grams, the *general* log-transformed regression equation would be adequate for use to approximate the leaf areas for grapes with a *moderately good* degree of accuracy. The application of this *general* equation would be enhanced if the area prediction for each *test* sample were performed on two or more replicates, and if the 90% upper and lower bounds were used in place of the point estimate.

REFERENCES

California Department of Food and Agriculture (1989) Reentry interval adjustments. California Code of Regulations Title 3, Chapter 6, Section 6774

Frear DEH (1935) Photoelectric apparatus for measuring leaf areas. Plant Physiol **10**:569-574

Furness GO, Newton MR (1988) A leaf surface scanning technique using a fluorescence spectrophotometer for the measurement of spray deposits. Pestic Sci **24**:123-137

Gunther FA, Westlake WE, Barkley JH, Winterlin W, Langbehn L (1973) Establishing dislodgeable pesticide residues on leaf surfaces. Bull Environ Contam Toxicol **9**:243-249

Gunther FA, Barkley JH, Westlake WE (1974) Worker environment research: II. sampling and processing techniques for determining dislodgeable pesticide residues on leaf surface. Bull Environ Contam Toxicol **12**:641-648

Iwata Y, Spear RC, Knaak JB, Foster RJ (1977) Worker reentry into pesticide-treated crops: I. procedure for the determination of dislodgeable residues on foliage. Bull Environ Contam Toxicol **18**: 649-653

Langer RHM (1956) Measurement of leaf growth in grass. In: Milthorpe FL (ed) The Growth of Leaves, Butterworths, London, pp.197-198

Nigg HN, Stamper JH, Queen RM (1974) The development and use of a universal model to predict tree crop harvester pesticide exposure. Am Ind Hyg Assoc J **45**:182-186

Popendorf WJ, Leffingwell JT (1982) Regulating OP pesticide residues for farm worker protection. Residue Rev **82**:125-201

Saiz S (1990) Predicting leaf surface area using mass-area relationships. HS No. 1546, The Worker Health and Safety Branch, California Department of Food and Agriculture

Sharp RB (1974) Spray deposit measurement by fluorescence. Pestic Sci **5**:197-209

Sharratt BS, Baker DG (1986) Alfalfa leaf area as a function of dry matter. Crop Sci **26**:1040-1042

Snedecor GW, Cochran WG (1980) Statistical methods. The Iowa State University Press, Ames, Iowa

Speelman LJ (1971) A fluorescent tracer technique for determination of the liquid distribution of field crop sprayers. J Agr Eng Res **16**:301-306

Stafford EM, Byass JB, Akesson NB (1970) A fluorescent pigment to measure spray coverage. J Econ Entomol **63**:769-776

Watson DJ (1937) The estimation of leaf area in field crops. J Agr Sci **27**:474-483
Westlake WE, Gunther FA, Carman GE (1973) Worker environment research: dioxathion (DELNAV®) residues on and in orange fruits and leaves, in dislodgable particulate matter, and in the soil beneath sprayed trees. Arch Environ Contam Toxicol **1**:60-83
Zweig G, Gao RY, Witt JM, Popendorf WJ, Bogen K (1984) Dermal exposure to carbaryl by strawberry harvesters. J Agr Food Chem **32**:1232-1236
Zweig G, Leffingwell JT, Popendorf WJ (1985) The relationship between dermal pesticide exposure by fruit harvesters and dislodgeable foliar residues. J Environ Sci Health **B20**:27-59

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