Vitis **41** (3), 137–142 (2002)

Discrimination of grapevine (*Vitis vinifera* L.) leaf shape by fractal spectrum

S. Mancuso

Dipartimento di Ortoflorofrutticoltura, Università di Firenze, Italia

Summary

A technique was developed (1) to digitise grapevine leaves, (2) to split the images obtained in the three components of the RGB (red, green and blue) colour system, obtain the fractal spectrum for each colour component of the leaf and (3) to calculate 15 different fractal parameters. The system, consisting of a scanning device, a personal computer and the code written in the C language, was then tested to characterize and identify 12 Sangiovese-related grapevine accessions. The results enabled us to distinguish between all accessions with a better discrimination than that obtained in previous studies with molecular markers or elliptic Fourier analysis. More, all the fractal parameters calculated for leaves of Sangiovese R10 grown in very different environments did not show any significant modification, revealing that fractal features can be considered environment-independent. The fractal analysis approach proposed, on the basis of the results obtained, gives the opportunity to verify the characters of distinction, uniformity and stability (DUS) requested by the Union for the Protection of Plant Varieties (UPOV) before plant breeders rights are granted.

K e y w o r d s : ampelography, fractal spectrum, cultivar identification, shape analysis, *Vitis vinifera.*

Introduction

The application of the fractal dimension to describe the structure of biological objects has been reported by several scientists (Logan and Wilkinson 1990; Moghaddam 1991; Avnir *et al*. 1992; Cox and Wang 1993; Anderson *et al.* 1996; Smith *et al.* 1996). Classic fractal analysis involves estimation of the perimeter of an object using rulers of different lengths. As the size of the measuring unit decreases, the estimated perimeter increases. These data, plotted as log of perimeter *versus* log of measuring unit are linearly codependent. This is known as the Richardson plot and the fractal dimension = D, where 1-D is the slope of the regression line. This is illustrated by the 'Coast of Britain' effect which, although reported by Lewis Richardson in the 1920s, was not widely known until the publication of Benoit Mandelbrot's seminal work, 'The Fractal Geometry of Nature' (Mandelbrot 1977).

Judging by the wealth of publications, the concept of fractal scaling is well understood and has been succesfully applied for the characterisation of structures and processes in plants (Mancuso 1999 a; Horgan 2001; Rice *et al*. 2001).

The classical approach in the description of complicated structures such as grapevine leaves relies on system decomposition into constituent simpler parts. This approach proved its limitations in many cases, when important properties emerge from the relations between the parts at different scales, between systems at different hierarchic levels. Fractal theory is specifically meant to approach structural relations, long range correlations in space and time, relations between hierarchic levels, in an effective way. In the case of the irregular shapes of grapevine leaves, simplicity acquired by assimilation with figures of Euclidean geometry would not do. Fractal theory is able to help capture the fingerprint of highly complex, irregular structures, paving the way to new horizons both in scientific research and in practical applications.

Mandelbrot (1977), in formulating the principles of fractal geometry illustrated that natural objects have a finite range over which they are approximately fractal curves and this was proved true also for grapevine leaves that exhibit a precise 'fractal range' (Mancuso 1999 a). Thus, the measuring units should range from the magnitude of the smallest feature of interest to the largest feature of interest. The range over which an object exhibits apparent self-affinity or self-similarity is determined by the structural and functional properties of the analysed structure. Therefore, it is imperative when estimating the fractal dimension that the size of the lower and upper limits of the structure have been determined. More, an object such as a leaf can be constituted by numerous fractal structures. In fact analysing the fractal geometry of a leaf and taking in consideration all the points of the leaf that show the same intensity of colour, will result in a fractal structure for each considered intensity of colour. Accordingly, if we want to characterize objects, like a grapevine leaf, as fractal, we don't know which part of colour information should be masked to form corresponding fractal. As a consequence we have to form all possible fractals, determine their fractal dimension and then examine fractal dimension as a function of the masking conditions. In other words, we have to create a fractal spectrum of the leaf.

The aims of this study are to devise a reproducible method for the calculation of the fractal spectrum of grapevine leaves and to show that the fractal spectrum can be

Correspondence to: Prof. Dr. S. Mancuso, Dipartimento di Ortoflorofrutticoltura, Polo Scientifico - Università di Firenze, Viale delle Idee 30, I-50019 Sesto F.no (FI), Italy. Fax: +39-055-4574017. E-mail: stefano.mancuso@unifi.it

used to discriminate grapevine leaves belonging to different genotypes using a backpropagation neural network in the analysis of the data.

Material and Methods

Plant material and image acquisition: The study was carried out with 11 putative Sangiovese-related ecotypes and the registered clone Sangiovese R 10 as reference (Tab. 1). The 12 accessions which were utilised in previous studies (Mancuso *et al*. 1998; Mancuso 1999 a, b; Mancuso 2001 a) and characterised by DNA marker technology (Sensi *et al*. 1996), were selected because they offered the possibility to verify the technique.

Table 1

Grapevine accessions of this study

#	Genotype
1	Prugnolo gentile
2	Brunellone
3	Brunelletto
4	Prugnolo acerbo
5	Prugnolo dolce
6	Prugnolo medio
7	Casentino
8	Chiantino
9	Morellino
10	Morellino di Scansano
11	Piccolo precoce
12	Sangiovese R 10

Samples were collected from the grapevine germplasm collection of the Department of Horticulture of the University of Florence. At veraison, from 15 plants per accession 65 fully expanded, healthy looking leaves, located between the 7^{th} and 11^{th} node (Allewelдt and Dettweiler 1986) were selected according to uniformity of appearance, growth habit and exposure. Leaves of the clone Sangiovese R10, originating from three very different sites in central and northern Italy were utilized to test the stability of the fractal dimension in relation to the environment.

Leaf images were acquired at 300 x 300 d.p.i., 16 million colours, by using an optical scanner.

Colour: All colours we perceive are determined by the response they produce in three retina cell types with well known spectral responses. Thus, most technological handling of colour (television, computer monitor, digital camera) imitates these three components with the familiar RGB (red, green, and blue) system. The three colours are combined in various proportions to produce all the colours displayed on the screen. They are referred to as additive because combined they produce white. Primary colours are measured as values from 0 to 255. The colours produced by combining the three primaries are a result of the relative strength of each primary. For example, pure red has a red value of 255, a green value of 0, and a blue value of 0. Yellow has a red value of 255, a green value of 255, and a blue value of 0. The absence of the three primary colours results in black; when all three have values of 255, they produce white. Levels of R, G, and B can each range from 0 to 100 % of full intensity. Each level is represented by the range of decimal numbers from 0 to 255 (256 levels for each colour), equivalent to the range of binary numbers from 00000000 to 11111111. The total number of available colours is 256 x 256 x 256, or 16,777,216 possible colours.

Thus a full representation of the colour of an object requires just the specification, for each single pixel, of the three-dimensional distribution (R,G, B).

In the present work each leaf image was (1) splitted in the three constituting channels, (2) each channel was thresholded for a colour value between 0 and 255 and (3) the fractal dimension for each colour value was calculated. Fig. 1 exemplifies the different phases of the fractal analysis performed.

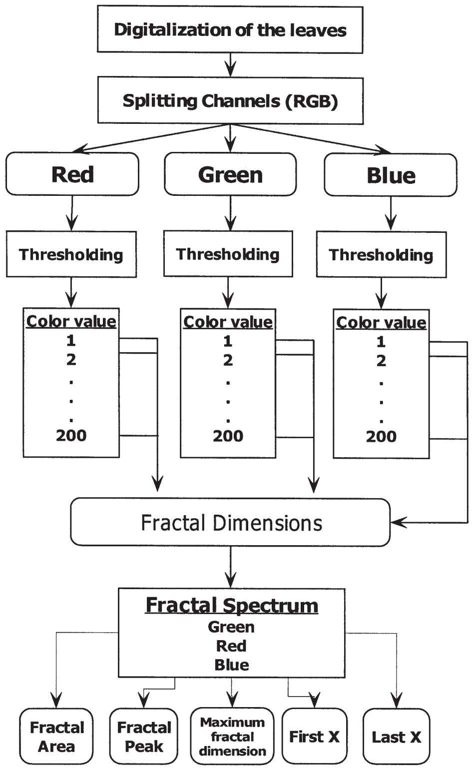

Fig. 1: Diagram of the operations.

Fractal dimension and fractal spectrum: Fractal dimension was assessed using the box-counting method. The implementation of these methods has been described in detail by Dennis and Dessipris (1989) and Mancuso (1999 a). In brief, the typical technique for determination of the BCD consists in partitioning the image space in boxes of size d x d and counting the number N(d) of boxes that contain at least one part of the shape to be investigated. Several values of d are chosen and the least square fitting of log[N(d)] x log(d) is used to determine the value of BCD. However, this approximation will suffer from effects caused by spatial quantization as well as the limited fractality of most natural objects (such as grapevine leaves). Therefore, the curve log[N(*d*)] x log(*d*) will exhibit two distinct regions. The error is minimised by calculating D in the region where the curve is most linear. Such guidelines were applied in the present research on grapevine leaves to obtain their Ds.

The fractal dimension was calculated and plotted against the colour intensity to obtain the fractal spectrum for the three channels red, green and blue. A baseline was drawn corresponding to the fractal dimension of 1 (by definition an object is a fractal just for values of the fractal dimension higher than 1) and 5 parameters were calculated. Fig. 2 shows the parameters considered.

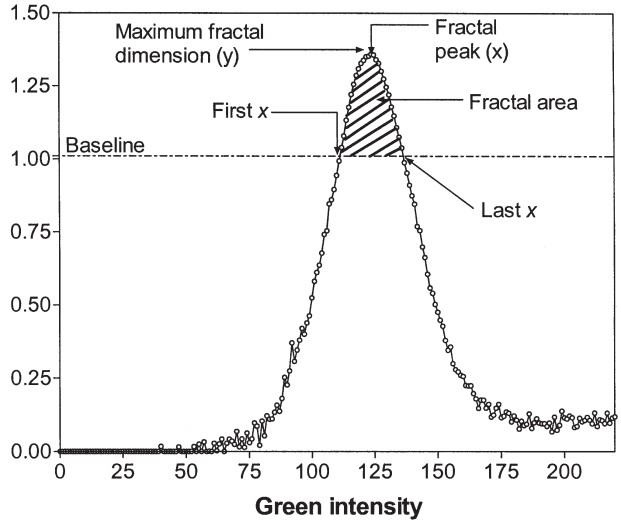

Fig. 2: Graphical representation of the 5 fractal parameters calculated from each colour channel. In this example the 5 parameters are calculated from the green channel spectrum.

N e u r a l n e t w o r k s : A back-propagation neural network (BPNN) program was written and implemented in a personal computer, following the methods previously described in MANCUSO *et al.* (1999). In brief, the networks were designed using a total of 15 inputs represented by the fractal analysis parameters (5 parameters for each colour channel). 12 outputs, represented by the accessions under examination, were used. In order to optimize the neural network activity, the number of 'hidden neurons' was modified. Minimum error was reached with 25 hidden neurons positioned on one level. The activation function of the neurons was a sigmoidal function, $1/(1+e^{-x})$. Back-propagation of error was performed using formulas previously described by MANCUSO and NICESE (1998). Details in back-propagating errors can be found in MANCUSO (2001 b).

The learning phase in all the BPNNs tested was protracted until the RMS (root mean square) error was <0.06 and the difference between the RMS in two consecutive epochs was <0.0001. The ANNs were tested with sets of fractal parameters in inputs for which the output was known, so that the predicted and actual outputs could be compared. These data had not been used previously to train the network.

Results and Discussion

Fig. 3 shows a characteristic example of the spectra of the three colour channels obtained from each leaf. The first spectrum from the right-hand side characterizes the properties of the leaf in the blue channel, whereas the second and third spectrum in the graph reflects the properties of the red and green channels, respectively. The baseline drawn for a value of the fractal dimension of 1 separates the fractal (>1) from the non-fractal (<1) zone of the spectrum. The parameter *fractal peak* is linked in to the shade of colour. If the position of the peak is close to zero, the total shade of the colour channel is darker. The parameter *fractal area* corresponds, in some way, to the cover ability of the colour channel and represents the total "fractality" of the leaf.

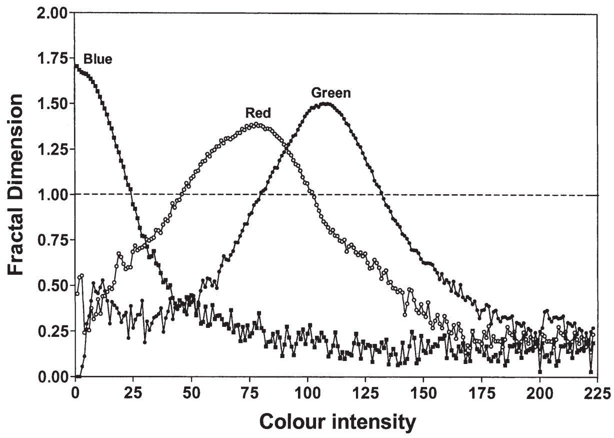

Fig. 3: Example of the appearance of the fractal spectra for the red, green and blue channel of a single grapevine leaf.

The mean values of the fractal parameters of a homogeneous sample of leaves from the clone Sangiovese R10, utilized as example, are reported in Tab. 2. The average standard error for the 5 fractal parameters studied was under 1 % (n=65). According with previous work showing, for the fractal dimension of the leaves, a very small variability among plants (MANCUSO 1999 a), the fractal parameters demonstrate a variability that is much smaller than the variability obtained with traditional ampelographic parameters. Moreover, the fractal parameters measured in leaves of cv. Sangiovese R10 grown in very different Italian environments showed no significant

T a b l e 2

Fractal parameters of homogeneous sets of leaves in the clone Sangiovese R10

	Green	S.E.	Red	S.E.	Blue	S.E.
Fractal area	15.85	0.03	13.78	0.05	10.14	0.08
Fractal peak	107.00	1.51	78.00	1.11	1.00	0.00
MFD*	1.50	0.00	1.39	0.00	1.72	0.00
First X	79.67	0.82	45.91	0.40	1.00	0.00
Last X	132.40	1.31	102.62	1.10	24.36	0.60

* Maximum Fractal Dimension

difference. A summary of results of this experiment is given in Tab. 3. The fractal parameters of the leaves are really stable in different environments. These results, also confirmed with other genotypes (data not published), lead us to consider the fractal parameters to be environment-independent.

To assess the usefulness of fractal measures in the task of automated plant identification from their leaves, a back-propagation neural network was designed and trained for the specific job of discrimination among different grapevine accessions. As input the BPNN was designed to use the 15 fractal parameters derived from the fractal spectra of the leaves. Tab. 4 shows the outputs of the recognition phase of the BPNN. Each row illustrates the ANN output for inputs represented by the fractal parameters of 15 leaves of a given accession. The neural network was able to easily discriminate among all the unknown accessions. Some accessions showed high output values also in columns different from the correct one. For example, in Prugnolo medio despite of the higher value (0.60) of the BPNN recognition phase output resulted in the correct column (Prugnolo medio), we have another high value of output in the column of the Prugnolo dolce (0.32). In this case the output shows a similarity between two accessions.

Comparing the present results with the results obtained with the same genetic material by PCR-based marker technologies (Sensi *et al.* 1996) or with elliptic Fourier analysis (EFA) (Mancuso 1999 b) demonstrates a higher capacity of fractal parameters in the distinction of different grapevine accessions. In fact, all the accessions were clearly differentiated in the present work, whereas (1) the accessions Morellino di Scansano, Prugnolo gentile and Sangiovese R10 were not distinct with PCR-based marker technologies (Sensi *et al.* 1996), and (2) Chiantino and Brunelletto were not distinct by EFA (Mancuso 1999). Moreover, a high degree of information can be achieved with the fractal measures if compared with the parameters derived from EFA. This means that with a minor number of fractal features, compared with EFA, it is possible to have a better or even complete discrimination.

Starting from the RGB colour system many parameters measuring differences or similarities between two images can be obtained. For example, the cumulative distribution F(R,G, B) or the proportion of pixels with a given red or green or blue value. Working on the discrimination of Brussels sprouts, Horgan *et al.* (1995) achieved a good discrimination between varieties using the mean value of R, G and B together with the proportion of pixel for which G>200 and for which G>225. The results obtained in the present work are encouraging as they demonstrate that the fractal spectrum carries all the information useful to discriminate different grapevine accessions. In other words, the fractal spectrum offers a unique quantitative framework for integrating all the information on colour, complexity and shape necessary to describe a grapevine leaf.

In conclusion, fractal parameters seem to be a useful tool for the identification of grapevine accessions on the basis of quantitative ampelographic traits. Therefore, this image analysis-based technique could be easily used for plant breeders right purposes, providing the opportunity to verify the characters of distinction, uniformity and stability (DUS) requested by the Union for the Protection of Plant Varieties (UPOV) before plant breeders right are granted.

Table 3

Effect of different environmental conditions on the fractal parameters in leaves of Sangiovese R10

Colour	Parameter	Tuscany	Umbria	Veneto
G	Fractal area	15.85 ± 0.03	15.61 ± 0.02	15.9 ± 0.02
r	Fractal peak	107.00 ± 1.51	106.21 ± 1.66	105.32 ± 1.96
e	MFD*	1.50 ± 0.00	1.49 ± 0.00	1.51 ± 0.00
e	First X	79.67 ± 0.82	77.3 ± 0.44	78.3 ± 0.78
n	Last X	132.40 ± 1.31	131.5 ± 1.6	132.3 ± 1.20
	Fractal area	13.78 ± 0.05	13.25 ± 0.03	13.66 ± 0.00
R	Fractal peak	78.00 ± 1.11	77.6 ± 1.23	77.32 ± 1.11
e	MFD*	1.39 ± 0.00	1.37 ± 0.00	1.26 ± 0.00
d	First X	45.91 ± 0.40	44.23 ± 0.33	43.20 ± 0.56
	Last X	102.62 ± 1.10	102.3 ± 1.52	101.9 ± 1.36
B	Fractal area	10.14 ± 0.08	9.98 ± 0.09	9.75 ± 0.11
l	Fractal peak	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
u	MFD*	1.72 ± 0.00	1.65 ± 0.00	1.56 ± 0.00
e	First X	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
	Last X	24.36 ± 0.60	23.63 ± 0.47	23.22 ± 0.72

* Maximum Fractal Dimension

Table 4

Averaged output of the back-propagation neural network (BPNN) recognition phase. Each row shows the BPNN output for the input represented by the fractal parameters of 15 leaves. The accessions are identified if the output which present the highest value (closest to 1) is in the correct column (with the same name)

Genotype	Brunelletto	Brunellone	Casentino	Chiantino	Morellino	Mor. Scans.	Piccolo precoce	Prugnolo acerbo	Prugnolo dolce	Prugnolo gentile	Prugnolo medio	Sangiovese R10
Brunelletto	**0.75***	0.02	0.00	0.02	0.03	0.01	0.00	0.00	0.00	0.02	0.00	0.05
Brunellone	0.03	**0.85**	0.00	0.00	0.00	0.01	0.00	0.04	0.01	0.04	0.00	0.02
Casentino	0.00	0.10	**0.71**	0.03	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.01
Chiantino	0.00	**0.33**	0.01	**0.56**	0.12	0.00	0.06	0.02	0.10	0.00	**0.41**	0.02
Morellino	0.01	0.00	0.00	0.01	**0.79**	0.16	0.00	0.00	0.00	0.00	0.00	0.00
Morellino Scans.	0.00	0.01	0.00	0.00	0.00	**0.68**	0.00	0.00	0.25	**0.27**	0.00	0.01
Piccolo precoce	0.06	0.00	0.02	0.05	0.00	0.18	**0.70**	0.00	0.00	0.02	0.01	0.08
Prugnolo acerbo	0.00	0.02	0.00	0.09	0.05	0.00	0.00	**0.76**	0.00	0.01	0.12	0.01
Prugnolo dolce	0.12	0.00	0.02	0.05	0.00	0.02	0.03	0.00	**0.66**	0.06	0.13	0.04
Prugnolo gentile	0.22	0.01	0.06	0.02	0.11	0.10	0.00	0.08	0.21	**0.55**	0.00	0.01
Prugnolo medio	0.00	0.00	0.00	0.02	0.01	0.07	0.05	0.22	**0.32**	0.00	**0.60**	0.03
Sangiovese R10	0.01	0.08	0.00	0.00	0.00	0.02	0.00	0.05	0.03	0.00	0.00	**0.89**

* The output with the highest values in each row are indicated in bold.

References

ALLEWELDT, G.; DETTWEILLER, E.; 1986: Ampelographic studies to characterize grapevine varietes. 4° Simp. Intern. di Genetica della Vite, Verona. Vignevini **13** (12, suppl.), 56-59

ANDERSON, A. N.; MCBRATNEY, A. B.; FITZPATRICK, E. A.; 1996: Soil mass, surface and spectral fractal dimensions estimated from thin section photographs. American Journal of Soil Science Society **60**, 962-969.

AVNIR, D.; FARIN, D.; PFEIFER, P.; 1992: A discussion of some aspects of surface fractality and of its determination. New J. Chem. **16**, 439-449

COX, B.; L. WANG, J. S. Y.; 1993: Fractal surfaces: Measurement and applications in the earth sciences. Fractals **1**, 87-115.

DENNIS, T. J.; DESSIPRIS, N. G.; 1989: Fractal modelling in image texture analysis. IEEE Proc. **136**, 227-235.

HORGAN, G. W.; 2001: The statistical analysis of plant part appearance – a review. Comp. Electron. Agric. **31**, 169-190.

HORGAN, G. W.; TALBOT, M.; DAVEY, J.; 1995: Plant variety colour assignment using a still video camera. J. Plant Var. Seeds **8**, 161-170.

LOGAN, B. E.; WILKINSON, D. B.; 1990: Fractal geometry of marine snow and other biological aggregates. Limnol. Oceanogr. **35**, 130-136.

MANCUSO, S.; 1999 a: Fractal geometry-based image analysis of grapevine leaves using the box counting algorithm. Vitis **38**, 97-100.

MANCUSO, S.; 1999 b: Elliptic Fourier analysis and artificial neural networks for the identification of grapevine (*Vitis vinifera* L.) genotypes. Vitis **38**, 73-77.

MANCUSO, S.; 2001 a: Clustering of grapevine (*Vitis vinifera* L.) genotypes with Kohonen neural networks. Vitis **40**, 59-63.

MANCUSO, S.; 2001 b: Frattali e reti neurali artificiali. Proc. Accad. Georgofili, Firenze (in press).

MANCUSO, S.; FERRINI F.; NICESE, F. P.; 1999: Chestnut (*Castanea sativa* Mill.) genotype identification: An artificial neural network approach. J. Hort. Sci. Biotech. **74**, 777-784.

MANCUSO, S.; NICESE, F. P.; 1999: Identification of olive (*Olea europaea* L.) varieties using artificial neural networks. J. Am. Soc. Hort. Sci. **124**, 527-531.

MANCUSO, S.; PISANI, P. L.; BANDINELLI, R.; RINALDELLI, E.; 1998: Application of an Artificial Neural Network (ANN) for the identification of *Vitis vinifera* L. genotypes. Vitis **37**, 27-32.

MANDELBROT, B. B.; 1977: The Fractal Geometry of Nature. W. H. Freeman, New York.

MOGHADDAM, B.; HINTZ, K. J.; STEWART, C. V.; 1991: Dimension and lacunarity measurement of IR images using Hilbert scanning. International Society for Optical Engineering (SPIE) Proceedings Series **1486**, 115-126.

OIV-IBPGR-UPOV; 1983: Code de caractères descriptifs des variétés et espèces de *Vitis*. Bull. O I V (Off. Int. Vigne Vin), Paris.

RICE, S. K.; COLLINS, D.; ANDERSON, A. M.; 2001: Functional significance of variation in briophyte canopy structure. Am. J. Bot. **88**, 1568-1576.

SENSI, E.; VIGNANI, R.; ROHDE, W.; BIRICOLTI, S.; 1996: Characterization of genetic biodiversity with *Vitis vinifera* L. Sangiovese and Colourino genotypes by AFLP and ISTR DNA marker technology. Vitis **35**, 183-188.

SMITH, T. G.; LANGE, G. D.;. MARKS, W. B.; 1996. Fractal methods and results in cellular morphology-dimensions, lacunarity and multifractals. J. Neurosci. Methods **69**, 126-136.

Received April 4, 2002