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Fractal geometry-based image analysis of grapevine leaves using the box counting algorithm

by

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S u m m a r y : An image analysis method based on the box counting algorithm was evaluated for its potential to characterize grapevine leaves. Although vine leaves lack the self-similarity of the theoretical fractals, leaves are candidates for characterization using fractal analysis because of their highly complex structure. The results showed for 11 Sangiovese-related genotypes fractal dimensions significantly being different except for (1) Prugnolo acerbo, Prugnolo dolce and Prugnolo medio and (2) Chiantino, Brunelletto and Morellino di Scansano, which have shown a high similarity in agreement with previous studies. Fractal dimension calculated for leaves of Sangiovese R10 grown in very different environments did not show any statistically significant modification revealing that fractal dimension can be considered environment-independent. Consequently, fractal dimension could be used as a descriptive, scale-invariant, condensed, morphological parameter in ampelographic research. The role of the fractal dimension as an additional morphological parameter in future ampelographic classification schemes of grapevine leaves is discussed.

K e y w o r d s : ampelography, box-counting, cultivar identification, fractal geometry, Vitis vinifera.

Introduction

In spite of the fact that human beings can readily recognize a multitude of known shapes and even describe unknown objects based on analogies, shape analysis has turned out to be a difficult task to be implemented. The main causes of such difficulties are related to the poor knowledge of the actual shape analysis mechanisms employed by biological systems, as well as the limited performance of the existing computational shape theories with respect to general and real data conditions. The ability to correctly characterize shapes has become particularly important in biological sciences, where morphological information about the specimen of interest can be used in a number of different ways such as for taxonomic classification and research on morphology-function relationships.

Morphological leaf characters and quantitative measurements of anatomical elements of the leaf, *i.e.* angles, area, teeth number, petiole length, have been extensively utilised in ampelographic research (OIV-IBPGR-UPOV charts 1983; Galet 1985). However, the origin of the varieties, their heterogeneity and the frequent cases of homonymy and synonymy, often resulted in doubtful classification. It is thus important to define good shape measures that can be effectively applied to leaf shapes, so they can be compared and analysed by meaningful and objective criteria. One approach that researchers have proposed for describing biological shapes is the fractal-based measure of digitally acquired images.

In both, natural and technical systems, objects can be characterized by fractional exponents describing their structure and behavior in one, two, or three Euclidean dimensions. These Euclidean dimensions do not adequately describe the morphology and behavior of the complex objects and relationships which are found in practice. Fractals are used to characterize, explain, and model complex objects in natural and man-made systems (Mandelbrot 1983).

Fractal geometry is concerned with geometric scaling relationships and the symmetries associated with them (Meakin 1988). Definitions of fractals are often influenced by the fields in which they are applied. However, all fractal structures share the following three characteristics: (1) self-similarity, (2) expression of a power-law relationship between two variables, (3) characterization by a non-integer fractal dimension

Self-similarity can be defined as invariance in the geometric properties of an object under isotropic rescaling of its lengths (Vicsek 1992). Jullien and Botet (1987) described fractals as "a rugose object whose rugosities show up at any length scale". A commonly used analogy are the Russian nested dolls in which each part of the structure is a copy of that structure (McWhinnie 1995). Fig. 1 demonstrates self-similarity using two conceptual fractal objects, the fractal gasket and the Menger sponge.

The second characteristic of fractals is that they express a power-law relationship between two variables. For example, the mass-to-length relationship of a fractal object can be expressed by

$$M \propto L^D,$$

where D is the fractal dimension (Li and Ganczarczyk 1989). The power-law behavior of the mass-size and density-size relationships is a function of the fractal's self-similarity (Mandelbrot 1983). This property of fractals allows them to be used extensively in modelling the structure and behavior of natural objects.

Finally, the fractal dimension, D, is a non-integer value in contrast to objects that lie strictly in Euclidean space (Akashi *et al.* 1994). In fractal theory, an irregular geometric is considered a transition between two regular ones.

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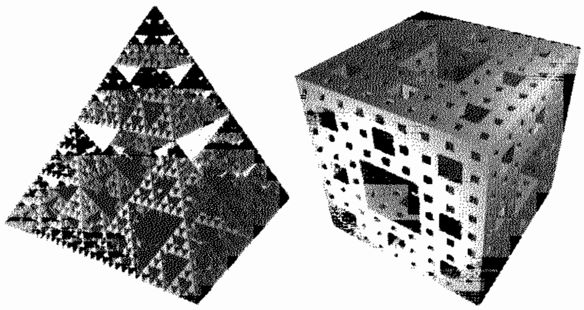

(a) (b)

Fig. 1: Conceptual fractal objects displaying self-similarity: (a) Fractal Gasket and (b) Menger Sponge.

A fractal curve (*e.g.* the Koch Triadic Curve) has a fractal dimension between a straight line and a plane (1 < D < 2), while a 3D fractal object (*e.g.* fractal gasket in Fig. 1) has a dimension between a plane and 3D space (2 < D < 3).

Fractal geometry-based analysis has received increasing attention as a number of studies have shown fractal-based measures to be useful for characterising complex biological structures in human anatomy (Caldwell *et al.* 1990, Glenny *et al.* 1991, Glenny and Robertson 1991) or in many fields of engineering and science (Logan and Wilkinson 1990, Moghaddam 1991, Avnir *et al.* 1992, Cox and Wang 1993, Anderson *et al.* 1996, Smith *et al.* 1996). Fractal scaling is evident in natural objects from the micro-scale to the macro-scale, *e.g.* the human body contains many structures with fractal characteristics (Jelinek 1996). In fact, investigators have found that non-fractal objects were the exception, rather than the rule in many natural systems (Avnir *et al.* 1985).

Thus, it seemed interesting to verify the possible application of fractal analysis to describe grapevine leaves belonging to different genotypes with the aim to add an objective, clarifying dimension to the excessively convoluted field of ampelography.

Material and Methods

Plant material and image acquisition: The study was carried out with 11 putative Sangiovese-related ecotypes and the registered clone Sangiovese R10 as a reference (Tab. 1); the 11 ecotypes were recently characterized by DNA marker technology (Sensi *et al.* 1996) and by Elliptic Fourier Analysis (Mancuso 1999). Samples were collected from the grapevine germplasm collection of the Department of Horticulture of the University of Florence, Italy. At veraison, 50 fully expanded, healthy leaves from 15 plants per accession, located between the 7th and 11th shoot node from the apex (Alleweldt and Dettweiler 1986) were selected according to uniformity of appearance, growth habit and exposure. Leaves of the cv. 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 .

Table 1

Fractal dimension of homogeneous sets of leaves in different Sangiovese-related ecotypes

Genotype	Mean	S. E.	Minimum	Maximum
Prugnolo gentile	1.301	0.001	1.283	1.310
Brunellone	1.294	0.001	1.271	1.316
Brunelletto	1.230	0.004	1.202	1.274
Prugnolo acerbo	1.457	0.003	1.415	1.472
Prugnolo dolce	1.448	0.001	1.426	1.462
Prugnolo medio	1.468	0.001	1.444	1.482
Casentino	1.204	0.008	1.136	1.294
Chiantino	1.240	0.003	1.216	1.298
Morellino	1.278	0.001	1.262	1.315
Morellino di Scansano	1.246	0.004	1.225	1.302
Piccolo precoce	1.499	0.002	1.471	1.512
Sangiovese R10	1.372	0.001	1.353	1.389

Fractal analysis: Leaf images were scanned into the computer using a scanner which was connected to a P133 computer and driven by Adobe Image Processing Software. Fractal dimension was assessed using the box-counting method (Scion Image Release beta 3b). The implementation of these methods has been described in detail by Dennis and Dessipris (1989).

Five control images (Fig. 2) with known fractal dimensions were analyzed to test the effectiveness of the method. The steps of the box-counting algorithm are illustrated in Fig. 3. The original grayscale image (Fig. 3 a) is thresholded to create a binary image (Fig. 3 b), where a leaf is represented by black pixels. An edge detection algorithm is applied to the binary image to create an image containing only the edge of the leaf (Fig. 3 c). The edge image is divided into a grid of square subimages or "boxes" of fixed length, *d*, and the number of boxes containing part of an edge, N(*d*), is counted. N(*d*) is determined for a range of values of *d* (Figs. 3 d, e), and then the log[N(*d*)] *versus* log(*d*) is plotted. The most linear portion of the curve (shown as open circle in Fig. 3 f) is chosen and linear regression is performed on that segment of the curve. The box-counting dimension (BCD) is the negative of the slope of the regression line.

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 minimized 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.

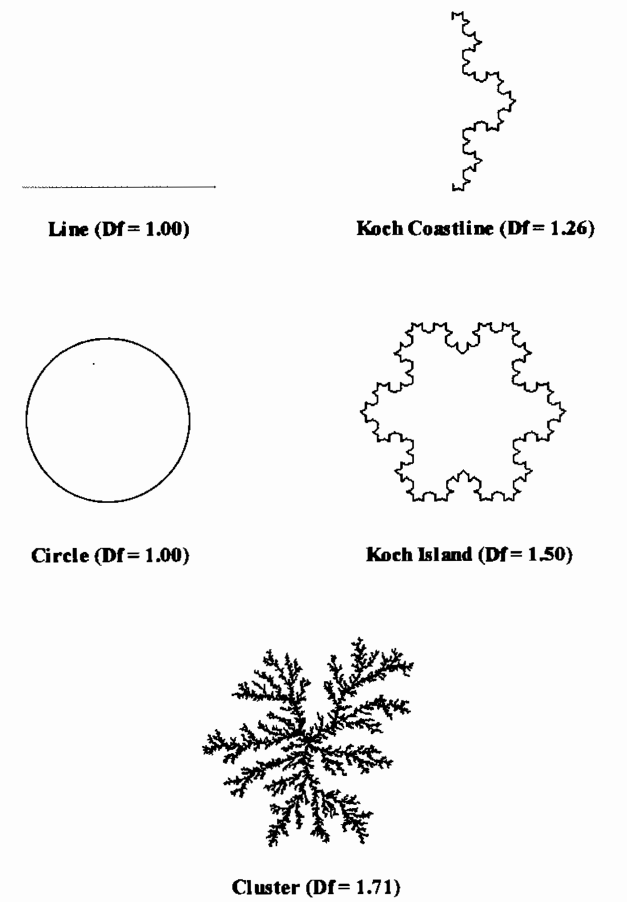

Fig. 2: Control images and their calculated/theoretical fractal dimensions (Df).

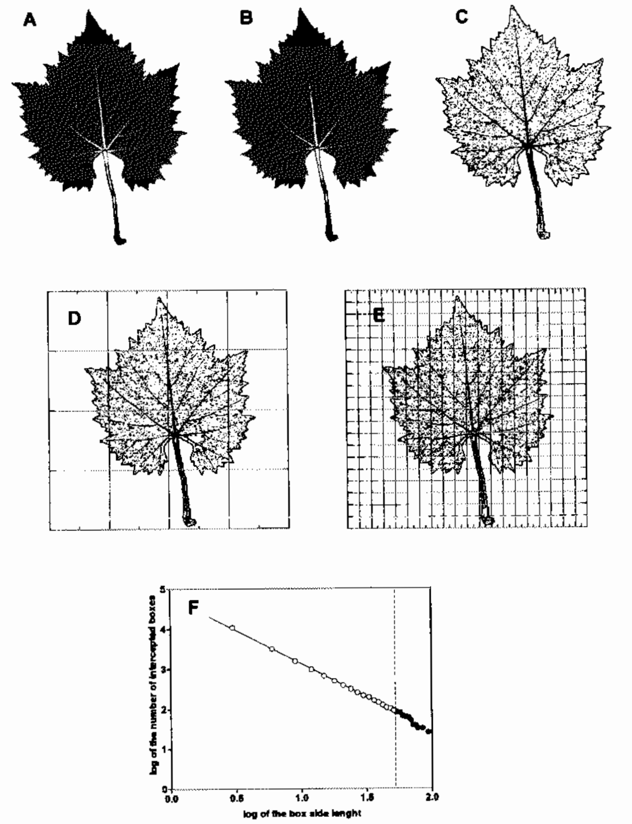

Fig. 3: Illustration of the steps involved in determining the BCD of an image showing the leaf shape as black pixels.

D a t a a n a l y s i s : All data derived from the fractal analysis were subjected to ANOVA using the program Statistica version 4.0 (Statsoft, Inc.).

Results and Discussion

The fractal dimensions of a homogeneous sample of leaves from different Sangiovese-related genotypes are listed in Tab. 1. The mean values of BCD ranged from 1.204 (Casentino) to 1.499 (Piccolo precoce), showing a rather ample interval. The mean values of D were statistically different for all the accessions except for 1) Prugnolo acerbo, Prugnolo dolce and Prugnolo medio and 2) Chiantino, Brunelletto and Morellino di Scansano which were statistically not distinguishable among them, but were different from all other genotypes. In both cases, results agree with studies based on molecular markers (Sensi *et al.* 1996) and on Elliptic Fourier Analysis and neural networks (Mancuso 1999), that showed a high degree of relatedness both, for Prugnolo acerbo, Prugnolo dolce, Prugnolo medio and for Chiantino, Brunelletto, Morellino. The results support the hypothesis that each of these two groups has been originated by mutation from an original seedling.

In spite of plant variability, the fractal dimension can be found quite accurately with a small sample size. The average standard error of D for 12 genotypes shown in Tab. 1, for example, was only 0.19 % (n = 50), that is much less than the standard error obtained with the traditional ampelographic parameters (Alessandri *et al.* 1996, Costacurta *et al.* 1996). Moreover, the fractal dimension calculated for leaves of Sangiovese R10 grown in very different environments in Tuscany, Umbria and Veneto did not show any statistically significant modification. Tab. 2 reveals that the variation in BCD due to changes in environment is small, if not absent, compared to the variation in BCD between different specimens. Although further research on other genotypes is necessary to assess the stability of the BCD in very different environments, the BCD can be considered to be somewhat environment-independent.

There is a number of fractal dimension estimation techniques (*e.g.* mass-radius method, parallel-line method, cumulative intersection method) that could have been used in leaf analysis. These methods including frequency-domain techniques as well as spatial-domain techniques such as the box-counting algorithm are currently evaluated for

T a b l e 2

Impact of different environments on the BCD (box-counting dimension) value in leaves of Sangiovese R10

Site	Mean	S. E.	Minimum	Maximum
Tuscany	1.372	0.001	1.353	1.389
Umbria	1.365	0.004	1.342	1.441
Veneto	1.383	0.002	1.326	1.415

their applicability in studying leaf structures. These studies will indicate the most proper methods to analyse living structures as leaves, which do not seem to show a perfect fractality. In fact, using fractal theory studies of natural objects have shown that true self-similarity is rarely observed in nature (Mandelbrot 1983; Kindratenko *et al.* 1994). Thus, it is a fundamental question on the applicability of fractal analysis to vine leaves if they are genuine self-similar objects. Results presented here show that leaves are not truly fractal because they do not show the highly hierarchical structure characteristic of artificial fractal object as the Fractal Gasket or the Menger Sponge showed in Fig 1. Nevertheless, the BCD gives an effective dimension that can be used to measure the complexity of highly complex structures such as vine leaves. Complex objects may show a power-law property over a limited range of scales and this property may be captured using fractal techniques (Pentland 1984, Dennis and Dessipris 1989). Similar discussions were met in the application of fractal analysis to other not truly fractal objects as the human trabecular bone or the neurons (Chung *et al.* 1994, Jelinek and Spence 1997). Consequently, this study rather than proposing that vine leaves are fractal, emphasizes the usefulness of fractal analysis in ampelography.

In conclusion, the results presented here show that fractal geometry can be utilized to analyze grapevine leaves. Within a limited range, leaves are self-similar with the mathematical characteristics of a fractal object. Taking into consideration that D seems to be insensitive to alterations of the morphology by environment, fractal dimension could be used as a descriptive, scale-invariant, condensed, morphological parameter in ampelographic research as well as in studies on the efficiency by which leaves fill the space in a canopy or to better understand shape changes during leaf ontogeny.

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