Viagra Induces Fractal Growth in Mushrooms in Switzerland

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This study investigates the effect of Sildenafil (a synthetic drug that is known to much of the public as "Viagra") on the growth and shape of the mushroom *Boletus edulis* (a Basidiomycete of the Boletaceae family), diffused in the beechwood trees (*Fagus*) of Ticino, the region with Italian culture located in the Southern part of Switzerland.

Background: About Sildenafil, and these Mushrooms, and Fractal Analysis

The pharmacological compound Sildenafil has been shown to treat the male erectile dysfunction "*impotentia erigendi* " by inhibiting a set of c-Phospodiesterase-5 enzymes, what in turn intensifies the blood afflux into the "corpora cavernosa", filling them up to turgidity leading to penis erection. (Dunzendorfer 2004).

Mushrooms of Boletaceae family including the *Boletus edulis* type, mostly display a phallus-like morphology formed by a stalk and a cap (pileus) whose extent and consistency may be influenced by various environmental agents, including growth factors and complex molecules such as polyphenols and other natural antioxidants.

Some analogies were noticed between Sildenafil and the chemical structure of natural polyphenols, flavonoids and many other cyclic compounds as rosmarinic acid abundant in macro-fungi, which exhibited an antioxidant free-radical scavenging activity (Guo et al. 2012). These morphological and physiological properties prompted us to investigate the role of Sildenafil on the proliferative capacity and on the morphological changes of Boletus type growing in the natural habitat of a Fagus forest.

Proper morphologic measures were obtained by means of an analytical approach based on the principles of fractal geometry developed by Mandelbrot (1982). These principles can be used to unravel irregular pattern of biological elements and complex physio-pathological phenomena that could not reliably be understood by means of conventional Euclidean geometry (which is more appropriate for describing ideal regular objects).

The term fractal (fractus i.e. fragmented) derives from the Latin verb *frangere* and means to break, to create irregular fragments. To be called fractals, biological and/or natural objects must fulfil a certain number of theoretic and methodological criteria including a high level of organization, shape irregularity, functional/morphological and temporal auto-similarity, scale invariance, iterative pathways and a non-integer peculiar fractal dimension [FD]. Whereas mathematical objects are invariant or self-similar over an unlimited range of scales, actual biological components and living structures are statistically self-similar entities iterated within a fractal domain called a "scaling window" (Losa 2012). Only within this scaling window can the object's fractal dimension be calculated. The fractal dimension is a numerical statistical measure that one can use to describe these kinds of irregular shapes and self-similar processes and, on all scales large and small, to correlate their shapes with the functional behaviors. This is a powerful tool for better understanding a good many kinds of biological tissues, both normal and pathological.

Fractal Geometry Principles

In nature, a fractal object is defined by its structural properties, namely by surface rugosity and/or absence of smoothness, form invariance or self-similarity, complexity and a fractal dimension [FD] usually greater than the topological dimension [DT]. The Richardson-Mandelbrot equation provides the mathematical basis for understanding geometric and spatial fractal structures, namely $L(\varepsilon)=N(\varepsilon).(\varepsilon)$ (1) where $L(\varepsilon)$ represents the length of the biological component under investigation, (ε) the unit length of measure and $N(\varepsilon)$ the number of unit lengths (ε) needed to cover the contour $L(\varepsilon)$. By a logarithmic substitution the above equation can be rewritten as: $\log[L(\varepsilon)/lo] = (1-D) \log[\varepsilon/lo]$ (2) This equation depicts a straight line with slope 1-D, from which the dimensional exponent D can be calculated to yield the numerical value of the fractal dimension [FD] (Losa & Nonnenmacher 1996).

Material and Methods

Two square domains, each with a surface area of 100 m^2 (10 m x 10 m side), were selected inside of a beech (*Fagus*) forest along the *line Insubrica* crossing the central zone of Ticino in southern Switzerland. Both domains located at convenient distance in order to avoid spatial overlapping or any other undesirable effect.

To the test domain we applied 50 mg of Sildenafil per square meter of surface area. This corresponds to a dose usually prescribed to human males and gauged with respect to the estimated median human body surface of 2 m^2 (Haycock 1978).

The control area received a placebo deprived of the active substance.

Active compound and placebo were dispersed through the forest domains distinctly delimited by a visible red ribbon.

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In both area of interest, measurements were performed in the afternoon at a fixed time (5 pm), just preceding the planned treatment which was repeated every two days during fourteen days. Experiments were conducted during the first two weeks of September, a time of year that is usually characterized by stable meteorological conditions with an external day temperature interval between 18-30°C. This period also featured an absence of moon — we chose this time so as to prevent malicious sarcastic popular comments evoked when scientific knowledge is missing.

All *Boletus* grown in untreated (control) and treated areas were counted and the numerical density [#B/m^2] established. Specimens from both area were selected according to a random systematic procedure usually applied in stereology, sagittal sections cut through the entire fungus and pictures taken at 10 X magnification projected onto a two-dimensional plane. Well-identifiable cap and stalk profile outlines were segmented from grey level-threshold images by means of Image J program and from these digital micrographs the fractal parameters determined by using the FANAL++ package. This analysis included subroutines for applying the box-counting method, implementation of the power law equation, data fitting, visualization of the true fractal domain called "scaling window", and delimitation by upper and lower limits [$\varepsilon_1, \varepsilon_2$] of a bi-asymptotic curve fitting in a double logarithmic plot from which a straight line can be drawn, its slope being used to evaluate the fractal dimension [FD]. Both scaling limits were defined by determining the widest interval [$\log \varepsilon_1$-$\log \varepsilon_2$] within the standard deviation of the estimated slope. The practical evaluation of the fractal dimension could be obtained by the box counting method based on counting of the non-empty boxes N at a variable grid length (ε) (Dollinger et al. 1998).

Results

Data from *Boletus* specimens collected in the domain exposed to Sildenafil showed a significant growth increase as expressed by the numerical density [#B/m^2], which ranged from 0.15 at time zero to 0.5 at day 14 of treatment, whilst it remained stable around 0.2 without significant changes in the control domain. A significant reduction of the fractal dimension FD from 1.22 to 1.11 was measured, what actually quantified the loss of irregularity or complexity occurring on both stalk and cap border outlines during the treatment period. These results contrasted with those recorded on *Boletus* samples found in the control domain, where both parameters i.e. the fractal dimension [FD] of stalk and cap border outlines and the numerical density [#B/m^2] remained rather constant along the investigation period, with a median value accounting for 0.15 and 1.22 for [#B/m^2] and FD respectively (Figure 1).
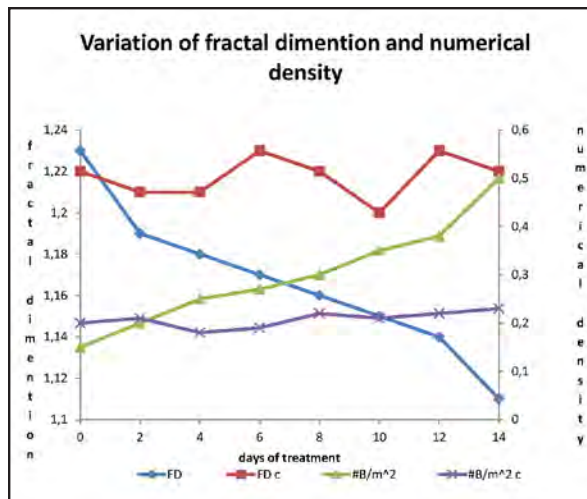

Figure 1. Variation of Fractal Dimension and Numerical Density in untreated and Sildenafil treated Boletus edulis. FD, fractal dimension of treated Boletus; FDc, fractal dimension of control Boletus; #B/m^2, numerical density of treated Boletus; #B/m^{2}c, numerical density of control Boletus.

Discussion

While the morphological, physiological and biochemical effects of Sildenafil on human male sexual apparatus are well documented, its action on *Boletus edulis* mushroom became clear only as a result of this study.

We found two quantifiable, notable effects of the drug on the mushrooms:

(A) a reduction of the fractal dimension [FD] which documented an increased smoothness of cap and stalk borders; and

(B) the increment of the numerical density.

Likely, the reduction of border irregularity is provoked by the stretching of cap surface and simultaneously, on the other, under side, by increased consistency or firmness of stalk tissue during growth. As far as is known, most biological processes governing growth and shape of fungi are not well understood — science still struggles to make complete sense of the complexity and specificity of their biological pattern. [For example, due to lack of chlorophyll, the fungus is unable to manufacture carbohydrates such as sugar, the backbone for the chemicals needed for life must be retrieved from the tissues of other organisms. Acids, iron, calcium, and other inorganic materials are derived from the soil, wood, or animal substances in which the mycelia grow, while not much fat was found nor starches were formed (Figure 2).] Compelling evidence has led to certainty that

drawing of water and basic food material occurs by the mycelium from the place where it grows.

Knowing the role of sildenafil on certain parts of the male human body, one can easily hypothesize an analogous effect on other, rather different biological targets such as *Boletus* mushrooms. According to such a hypothesis, in these mushrooms, a strengthened lymph drawing and water afflux suitable to permeate the roughage tissue, thus favoring both firmness of stalk and smoothness of cap. If so, then the effect induced in mushrooms might mimic the polymorphous effect observed in human males.

But how to explain the observed diverging behavior? On the one hand, the numerical density [#B/m^2] increased by 35%, proving a significant growth of *Boletus* after fourteen days of treatment. On the other hand, the height dimensions of specimens treated with Sildenafil were found smaller than those of control area, with data interval ranging between 6.5-8.1 cm and 7.4-9.6 cm respectively.

Hence in this experimental system the growth rate was inversely related to *Boletus* height. The fractal dimension values recorded on the cap and stalk border outlines of *Boletus* mushrooms deserved a critical comparison with data recovered in the living realm; in the former fractal dimension values ranged between 1.10 and 1.23 rather close to fractal dimension values recorded on contour profiles of most biological structures and cell tissues, notably liver cells, healthy lymphoid and white blood cells, leukemic circulating cells, oocytes, immature astrocytes and neuronal cells, all characterized by a similar degree of irregularity (Losa & Nonnenmacher 1996).

Unfortunately, fractal dimension data on mushrooms have never been calculated (or at least have not been reported in the scientific literature).

To sum up: The numerical density (#B/m^2) of the mushroom was increasing with time, i.e. from 0.15/m^2 up to 0.5/ m^2 at day 14 of treatment, while the fractal density reduced from 1.23 to 1.11. Thus, there was a significant reduction of border profile complexity and irregularity in *Boletus* mushrooms that were treated with Sildenifil, a drug that provokes penile erections in human males.

Conclusion

To put this in simple terms:

Our investigation highlighted the main fractal principle which rests on the unlimited iteration of a unit fragment as a chief generator, either determined or unknown, until completion of the whole structure. The same principle serves to explain the fractality of growth mechanisms, the irregularity of morphological structures and the complex dynamics of living processes which occur at different spatial and temporal scales in connection with the principle of the recursive genome function (Pellionisz 2008), all the phenomena implicated in growth and maintenance of the fascinating and mysterious kingdom of mushrooms.

Figure 2. Boletus edulis: *color picture of autumnal specimens collected as indicated in the Material and Methods section.*

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