

***Ambrosia artemisiifolia* L. leaves fractal geometry variation in relation to nitrogen rate**

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Abstract

Common ragweed (*Ambrosia artemisiifolia* L.) plants grew and developed after harvesting experimental variants in wheat crop fertilized at variable nitrogen rates. Differential plant growth was observed, positively associated with nitrogen rate. The leaf geometry of common ragweed plants was evaluated by fractal analysis. The fractal dimensions (D) showed an increasing variation, in the range $D = 1.5741$ at the N0 level, up to $D = 1.7168$ at the N200 level. A polynomial equation described the variation of the fractal dimension (D) with nitrogen rate ($R^2 = 0.999$, $p = 0.0156$). Differences between the mean and median values of the D values, in the leaf samples on the variants N0, N50, N100, N150 and N200, were statistically confirmed. The variable rate of fractal dimension variation (D_{Vrv}) and the absolute rate of fractal dimension variation (D_{Arv}) based on leaf geometry were recorded, associated with the nitrogen levels on which common ragweed plants were grown. The fractal dimension (D) of ragweed leaf geometry can be a refined indicator that expresses the level of development, competitiveness and invasiveness potential of ragweed plants (*Ambrosia artemisiifolia*) in relation to the fertility level of the occupied lands, or potential to be occupied.

Keywords: *Ambrosia artemisiifolia*; comparative analysis; fractal; leaf geometry; nitrogen

Introduction

Fractal analysis is used in various scientific disciplines (e.g. biology, mathematics, medicine, ecology, economics, materials sciences, etc.) as an essential tool, with very high utility [11], [24], [17], [32].

In ecological studies, fractal analysis has been successfully used, promoted as a basic method, for the advantages of describing the geometry of very different natural objects as geometric shapes, to quantify the distribution of relevant shapes [34], [19], [13], [9].

For the study of plants, fractal analysis has been used to evaluate different organs, from roots, leaves, flowers, to whole plant [37], [1], [12]. Studies based on fractal analysis have considered the identification and classification of plant species in relation to geometric features quantified by fractal dimension [3], [2], [18]. The relationship of some plants with environmental factors was evaluated through fractal analysis, based on plant response traits, quantifiable in fractal dimension [4], [36].

Leaves are common organs in plants, with defining elements (shape, size, etc.) that vary greatly in relation to plant species, genotype, age, growing conditions, stress factors, etc. [5], [39], [30], [27]. Plant architecture, leaf size and shape vary in relation to nutrient factors, especially nitrogen [22], [35].

Fractal analysis has been used to analyze and characterize plant leaves, with different applicability and utility, both scientific and practical [25], [38], [7]. Based on the fractal geometry of leaves, plant species and genotypes (varieties, hybrids) were characterized for the purpose of their classification [40], [15], [18], [31]. Some studies have used fractal descriptors in the analysis of the geometry of leaf sections, obtained in microscopic images, for plant identification [6]. Spatial patterns of plants were determined through fractal analysis in relation to nitrogen and water regimes during the growing season [23].

Ragweed (*Ambrosia artemisiifolia* L.) is an invasive species (native to North America), widely distributed in Europe, America and Asia [43], [16], [21]. Influencing factors in relation to the ecological and physiological characteristics of the species were studied, in order to understand the invasive propagation and the potential areas for expansion and multiplication [45].

Among climatic factors, air temperature has been considered the important factor shaping the distribution of ragweed in different areas, and climate change favors the spread of ragweed in new areas [33], [16]. Soil characteristics, such as humidity, temperature and fertility, were influential factors in the propagation,

growth and development of ragweed, under different study conditions [28], [10], [8]. The relationship of ragweed at the rhizosphere level was analyzed in relation to the soil reaction, with nitrogen, as well as with various mineral elements in the soil [44], [21].

The study aimed to characterize, through fractal analysis, the geometry of common ragweed leaves (*Ambrosia artemisiifolia* L.) from plants grown after the wheat crop was harvested, on experimental wheat variants provided with different nitrogen rates.

Material and Method

The study was conducted at the Didactic and Experimental Resort, University of Life Sciences “King Mihai I” from Timisoara, in the agricultural year 2019 – 2020. In the wheat crop, the 'Ciprian' cultivar, an infestation with common ragweed (*Ambrosia artemisiifolia* L.) was recorded in the spring – summer of the 2020 year. After harvesting the wheat crop on experimental variants (beginning of July) the common ragweed plants continued to grow. The differentiated growth of ragweed plants on experimental variants was observed, with variable nitrogen fertilization rates for wheat.

At the beginning of August, leaf samples were taken from common ragweed plants, on the fertilization variants from the wheat crop experiment. The leaves were scanned at 1:1 ratio (HP CM2320fxi MFP). Examples of common ragweed leaves from the plants on variant sample set are shown in Fig. 1. Fractal analysis (box-counting) was performed on the binarized images, Fig. 2(a) [41], [29], under conditions of statistical safety ($R^2 = 0.998$), Fig. 2(b).

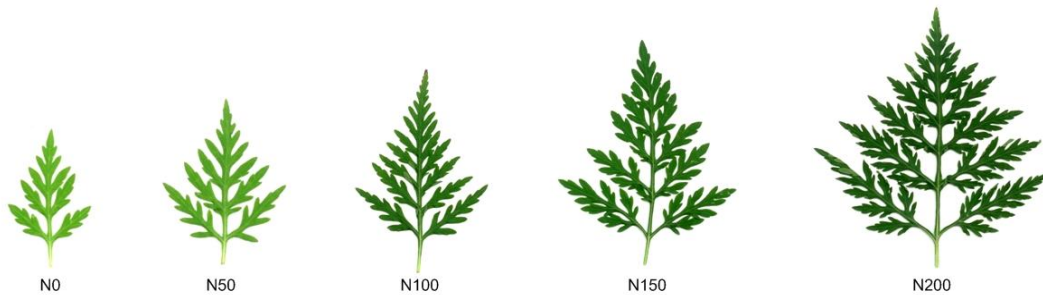


Figure 1. Examples of common ragweed leaves, from plants on plots with different nitrogen levels

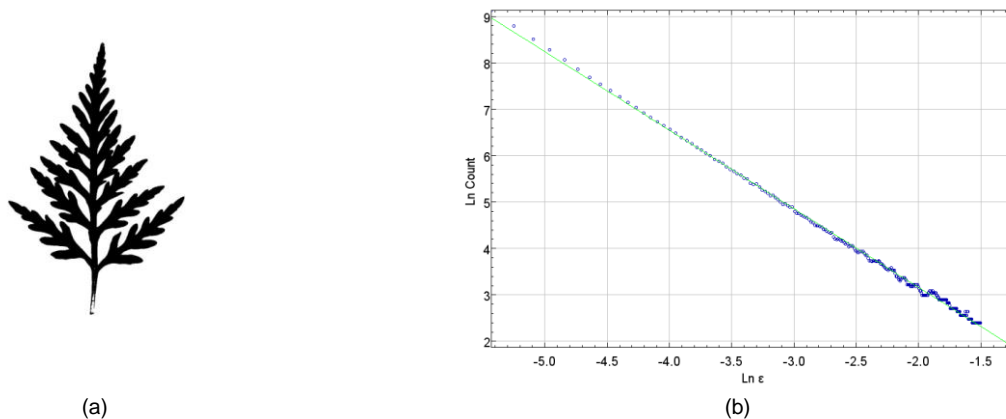


Figure 2. Fractal analysis of leaf geometry; (a) binarized image, (b) linear regression line

Fractal dimensions (D) were obtained to characterize the geometry of common ragweed leaves grown on different nitrogen levels. The data series were analyzed in terms of statistical reliability, the presence of variance (ANOVA Test). The level of differentiation of the mean and median values of fractal dimensions (D) was evaluated. Comparative analysis of the data was done by Mann-Whitney test (post-hoc step). The distribution trend of the data series was analyzed (Mann-Kendall test). Regression analysis was applied to evaluate the interdependence between the fractal dimension of the leaf geometry and the nitrogen novela on which the plants grew. PAST software v.4.17 [14] was used for statistical analysis of the data.

Results and Discussion

Scanned images of ragweed leaves were analyzed to obtain the fractal dimensions (D) of the leaf

geometry, at each nitrogen level. Fig. 3 shows the distribution of the fractal dimension (D) data series in box plot format, indicating the range of variation, median values and confidence interval.

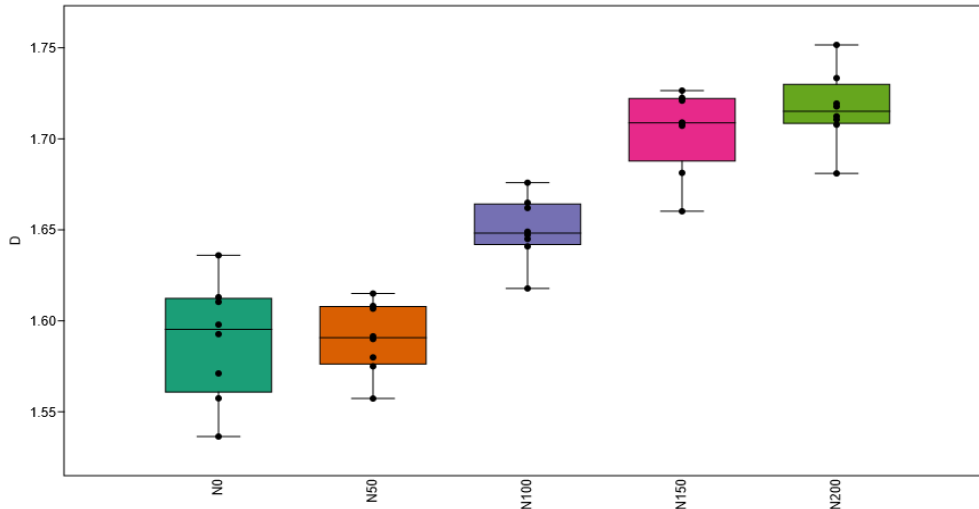


Figure 3. Graphical representation of D values in ragweed leaf samples

ANOVA Test indicated the statistical reliability of the experimental data, the presence of variance in the data set, and clear evidence for the inequality of the mean values of fractal dimension, across the data series in relation to nitrogen levels (Table 1).

Table 1. ANOVA Test results

Statistical parameters	Sum of sqrs	df	Mean square	F	p (same)
Between groups:	0.11722	4	0.02931	54.61	1.44E-14
Within groups:	0.01878	35	0.00054	Permutation (0.00000)	
Total:	0.13600	39	1.00E-05		
Components of variance (only for random					
Var(group):	0.00360	Var(error):	0.00054	ICC:	0.87016
omega2:	0.8428				
Welch F test in the case of unequal variances:	F=52.21, df=17.37, p=1.875E-09				
Bayes factor:	6.369E11 (decisive evidence for unequal means)				

The Kruskal-Wallis test indicated significant differences between the median values ($H(\chi^2) = 33.2$; H_c (tie corrected) = 33.2; $p < 0.001$), of the data series for fractal dimension (D) in ragweed leaves in relation to nitrogen levels. Based on the results of the ANOVA and Kruskal-Wallis tests, which indicated significant differences between the mean and median values calculated for fractal dimension (D), the nonparametric Mann-Whitney test (post-hoc step) was applied, Table 2.

Table 2. Mann-Whitney test results based on D values in common ragweed leaves

	D _{N0}	D _{N50}	D _{N100}	D _{N150}	D _{N200}
D _{N0}		0.8748	0.0014	0.0009	0.0009
D _{N50}	0.8748		0.0009	0.0009	0.0009
D _{N100}	0.0014	0.0009		0.0028	0.0009
D _{N150}	0.0009	0.0009	0.0028		0.4309
D _{N200}	0.0009	0.0009	0.0009	0.4309	

The data series on the fractal dimensions (D) of the geometry of ragweed leaves, on nitrogen (N) levels, was analyzed in terms of variation trend. Mann-Kendall trend test indicated an increasing trend, in

conditions of statistical safety, with the values of the test parameters, $S = 520$, $Z = 6.0469$, $p < 0.001$.

Regression analysis showed the trend of data distribution, with Slope = 0.0044676, intercept = 1.5587, $p(\text{slope}) < 0.001$, Table 3, with graphical representation in Fig. 4.

Table 3. Statistical parameters of Ordinary Least Squares Regression

Statistical parameters	Statistical parameter values			
Slope a:	0.004467	Std. error a:	0.00038	
	t:	11.683	p (slope):	3.82E-14
Intercept b:	1.5587	Std. error b:	0.008997	
95% bootstrapped confidence intervals (N=1999):				
Slope a:	(0.0036569, 0.0052069)			
Intercept b:	(1.5409, 1.5784)			
Correlation:				
r:	0.8844			
r ² :	0.7822			
t:	11.683			
p (uncorr.):	3.82E-14			
Permutation p:	0.0001			

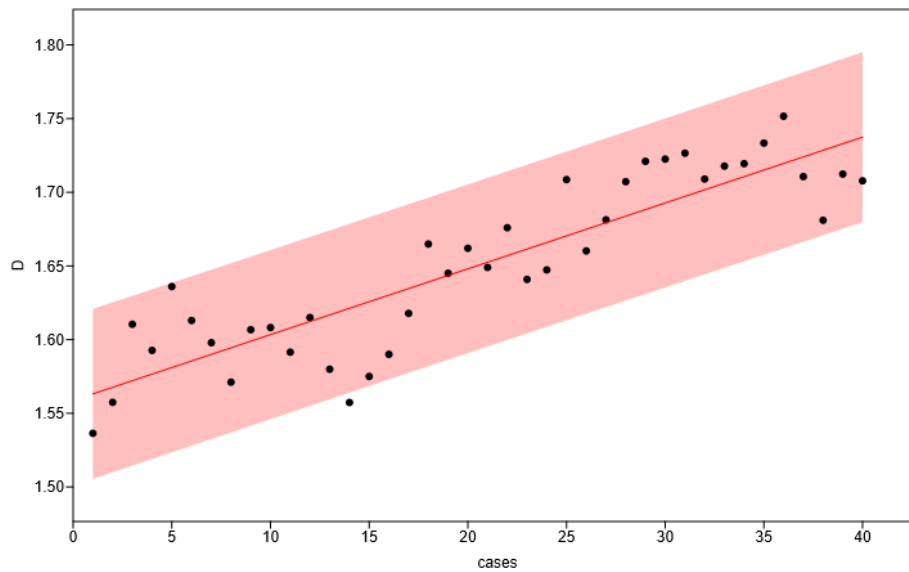


Figure 4. Linear fit model for fractal dimension values in common ragweed leaf geometry

The interdependence relationship between the fractal dimension (D) of the geometry of ragweed leaves, and the nitrogen levels on which the plants grew, was described by equation (1), with $R^2 = 0.999$, $F = 2202$, $p = 0.0156$, Fig. 5.

$$D = -5.687E-08x^3 + 1.666E-05x^2 - 0.0003438x + 1.574 \quad (1)$$

where: D – fractal dimension of common ragweed leaves; x – N level (kg/ha)

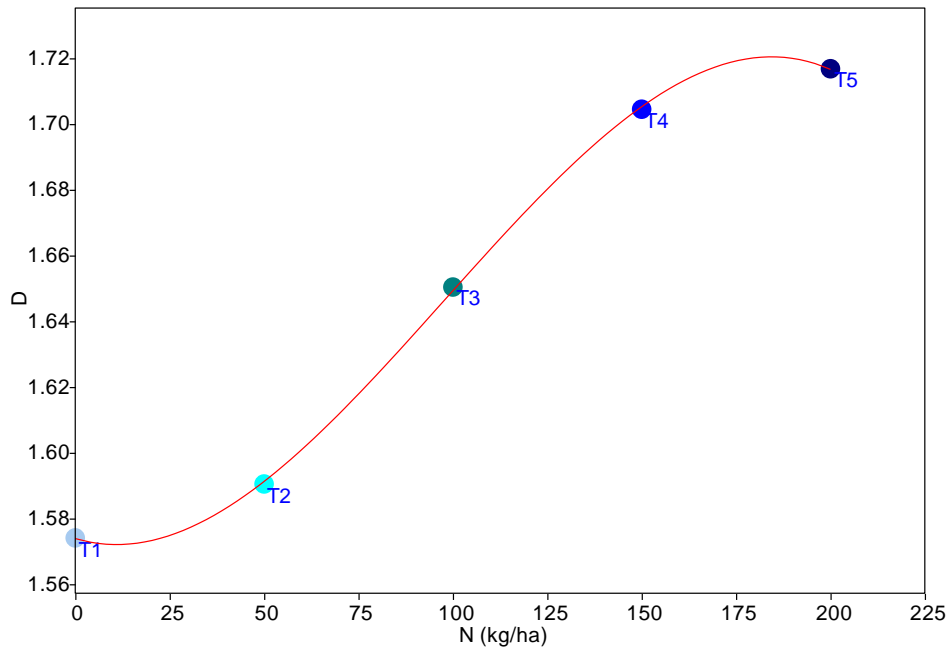


Figure 5. Graphical expression of the relationship between D and N on leaves in common ragweed plants (T1 – N0; T2 – N50; T3 – N100; T4 – N150; T5 – N200)

From the analysis of images of ragweed leaf samples, harvested from plants grown on different levels of nitrogen fertilization for wheat crops, leaf development was observed both in terms of dimensions (length, width) and number of branches, associated with the nitrogen rate.

Common ragweed positively utilized nitrogen, under experimental conditions, by increasing biomass production with the nitrogen rate [20].

Ambrosia artemisiifolia (invasive species) was tested against two native ragweed species (*Artemisia annua*, *Artemisia mongolica*) at three nitrogen rates. Under conditions of mixing the three species, *Ambrosia artemisiifolia* plants responded by significantly increasing with the nitrogen rate, while the other two species showed a low response, or even a decrease in growth rate with the nitrogen rate [42]. The authors of the study concluded that under conditions of high nitrogen levels, common ragweed (*Ambrosia artemisiifolia*) became a stronger competitor than the other two species. Nitrogen increased the competitive capacity of *Ambrosia artemisiifolia*, and may be a factor (nitrogen) facilitating the invasion of the species on high-fertility lands. The response of *Ambrosia artemisiifolia* plants to soil pH and nitrogen conditions has also been reported in other studies [44]. *Ambrosia artemisiifolia* was considered a nitrophilous plant species, with a favorable response to nitrogen in relation to the type of fertilizer, the applied rate and the type of soil [26], [21].

Fractal dimension expressed the variation of leaf geometry, and the variation factor was nitrogen level. Variable values of fractal dimensions were recorded, $D = 1.5894$ in the case of ragweed leaves from plants on the N0 level, $D = 1.5905$ in leaves on the N50 level, $D = 1.6504$ in leaves on the N100 level, $D = 1.7045$ in leaves on the N150 level and $D = 1.7168$ in leaves on the N200 level respectively. Fractal dimension showed the rate of variation (growth) of the geometry of common ragweed leaves, with the nitrogen rate.

Equation (1) described the variation of fractal dimension (D) in relation to nitrogen levels ($R^2 = 0.999$, $p = 0.0156$), and showed the close relationship between leaf geometry (quantified in fractal dimension D) and the nitrogen level on which ragweed plants grew. The result shows the ability of plants to capitalize on fertile soils, and the nitrophilic character of ragweed plants, confirmed by previous studies [26], [21].

The variable rate of fractal dimension variation – DVrv (Variable rate of variation – Vrv), Equation (2) and the absolute rate of fractal dimension variation DARv (Absolute rate of variation – Arv), Equation (3), for leaves geometry on different nitrogen rate, were calculated.

$$D_{Vrv} = D_N - D_{N-1} \quad (2)$$

where: D_{Vrv} - Variable rate of fractal dimension variation; D_N – fractal dimension of leaf geometry at the current N dose; D_{N-1} - fractal dimension of leaf geometry at the previous N dose.

$$D_{Arv} = D_N - D_{N0} \quad (3)$$

where: D_{Arv} - Absolute rate of fractal dimension variation; D_N - fractal dimension of leaf geometry at the current N dose; D_{N0} - fractal dimension of leaf geometry at the control N dose.

In the case of D_{Vrv} , the values recorded were $D_{Vrv} = 0.0164$ (N50), $D_{Vrv} = 0.0599$ (N100), $D_{Vrv} = 0.0542$ (N150), and $D_{Vrv} = 0.0122$ (N200). In the case of D_{Arv} , the values recorded were $D_{Arv} = 0.0164$ (N50), $D_{Arv} = 0.0763$ (N100), $D_{Arv} = 0.1305$ (N150), and $D_{Arv} = 0.1427$ (N200).

The D data series (fractal dimension) showed an increasing variation trend, confirmed by the Mann-Kendall test ($p < 0.001$).

The result showed the dependence between leaf geometry and the level of nitrogen supply of the soil. Ragweed plants exploited the N resource by increasing leaf size, increasing the variation of leaf geometry, as a positive response to the available nitrogen supply. The nitrophilic character of ragweed has been reported in previous studies [26], [21].

Comparative analysis of leaf geometry based on fractal dimensions according to Mann-Whitney test (post-hoc step), in relation to the nitrogen levels on which the plants grew, showed differences between leaf sampling variants, supplemented with variable levels of N from the wheat crop. The differences were statistically significant ($p < 0.01$; $p < 0.001$), except for the comparison between DN0 and DN50, respectively between DN150 and DN200 ($p > 0.05$).

Conclusions

Common ragweed (*Ambrosia artemisiifolia*) plants that grew after the wheat crop harvest utilized the nitrogen applied at variable rates to the wheat crop, through differentiated plant growth and development, closely dependent on the nitrogen rate. Leaf samples taken from plants that grew on the experimental wheat variants expressed the differentiated level of plant development.

Fractal analysis assessed the geometry of common ragweed leaves, and the fractal dimension D varied in close relationship with nitrogen levels applied to the wheat crop. A polynomial mathematical model described the variation of fractal dimension D with nitrogen rate ($R^2 = 0.999$, $p = 0.0156$).

Significant differences were recorded between the mean and median values (ANOVA Test, Kruskal-Wallis test; Mann-Whitney post-hoc step). Appropriate mathematical tests (Mann-Kendall; Regression analysis) showed the increasing trend of the data series (D), $p < 0.001$.

The fractal dimension (D) of the geometry of ragweed leaves can be an indicator of expression and estimation of the level of development, and of the potential for competitiveness and invasiveness of ragweed plants (*Ambrosia artemisiifolia*) in relation to the level of fertility (nitrogen rate) of the areas occupied, or potential to be occupied.

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