ANALYSIS OF *ARTEMISIA ARBORESCENS* L. GROWTH RATE ON CONTAMINATED SUBSTRATES USING A THERMODYNAMIC APPROACH.

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ABSTRACT

This article examines the growth rate of *Artemisia arborescens* L. on diversely contaminated artificial substrates, with a view to mine site remediation, adopting a thermodynamic approach.
Analysis of the experimental data on the growth of four cloned populations of the *Artemisia arborescens* L. species on three different artificial substrates, obtained by mixing different proportions of mineral matter and distilling slops, revealed several aspects of the phenomenon and a number of hypotheses have been advanced that warrant further investigation.
The findings of the experimental work confirm that *Artemisia arborescens* L, grows well on contaminated substrates improved with the addition of suitable quantities of organic matter

1. INTRODUCTION

In the preliminary feasibility evaluation of environmental remediation of mining areas it is important to determine the maximum threshold of contaminated material contained in the substrate, beyond which the partition function of growth rate ceases to be normal and unimodal. This threshold can prove a useful tool in planning and developing the projects in land degraded by anthropogenic pressure, which envisage planting indigeneous species following a time sequence that mimics natural processes known as successional stages

1. MATERIALS AND METHODS

The three growth substrates were prepared by mixing different proportions, as shown in Table 1, of flotation tailings from the derelict Montevecchio mine (CA) with pomace distilling slops produced by DI.CO.VI.SA srl (CA).

Table 1. Composition of substrates used in growth tests .

Substrate	Mineral fraction (% by volume)	Distilling slops (% by volume)
1	100	0
2	95	5
3	90	10

As far as the mineral fraction is concerned, mineralogical analyses showed the material to be composed chiefly of quartz (SiO_2), with subordinate phyllosilicate, presumably attributable to the illite, and minor amounts of siderite ($FeC0_3$).

Table 2. Chemical analysis of the three samples PL1, PL2, PL3 of flotation tailings from the Montevecchio mine.

XRF determinations

Major elements (% by weight)											
	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	K_2O	CaO	TiO_2	MnO	Fe_2O_3	FeO	Tot
PL3	0.34	0.79	12.61	64.5	0.09	2.98	0.13	0.37	0.35	11.81	0.68	99.95
PL2	0.33	0.78	12.13	67.17	0.09	2.94	0.14	0.35	0.53	10.78	0.24	99.98
PL 1	0.29	0.75	10.14	74.12	0.07	2.44	0.23	0.34	0.43	5.53	1.94	99.97

Minor elements (ppm)											
	Pb	Zn	Cd	Cu	Sb	Ni	Cr	Bi	As	Ag	Sn
PL3	3620	7400	24	296	115	33	75	<6	<5	<20	<20
PL2	2200	5840	25	211	82	29	54	<6	<5	<20	<20
PL 1	1300	5760	40	132	~9	33	49	6	<5	<20	<20

The bulk chemical composition (Table 2) agrees substantially with the mineral association: high silica content, corresponding to the quartz and illite, with some aluminum and potassium, attributable to the illite.
The bivalent iron (FeO) content is in good agreement with the small quantities of siderite, while the large proportions of trivalent iron (Fe_2O_3) can logically be assumed to be attributable to the presence of weakly crystalline ferric oxyhydroxides, typical of mining areas in oxidising environments ("mine ochres ").
All the other "major" elements are contained in such small quantities as to suggest the absence of other mineralogical phases. A small amount of magnesium (and in theory also Fe) could be contained in the illite as a vicariant of aluminum.
With regard to the potentially toxic heavy metals, the mineral matter examined here contains significant quantities of Zn and Pb (a few thousand ppm), and some traces (from tens to hundreds of ppm) of Cu, Ni, Cr, Cd, Sb.
The DI.CO.VI.SA slops are produced by a pomace distillery for alcohol production and thus contain neither industrial nor municipal residues. Prior to their use, they were screened through a 2mm sieve to obtain a sufficiently homogeneous material.
To minimise the effect of genetic factors, four clonal populations (A – B – C – D) were used obtained by asexual propagation taking cuttings from four mother plants growing in the natural environment at Capo S. Elia near the city of Cagliari.
Each clonal population, each comprising 75 individuals, was raised on 3 substrates (Table 1), as shown in Table 3.

Table 2. Scheme of growth test.

	SUBSTRATE 1	SUBSTRATE 2	SUBSTRATE 3
CLONE A	1/A	2/A	3/A
CLONE B	1/B	2/B	3/B
CLONE C	1/C	2/C	3/C
CLONE D	1/D	2/D	3/D

2. RESULTS

The experimental data consist of the height reached by each individual, measured at time intervals, and then calculating the mean value for each test.

From the graphs shown in Figure 1, which depict growth dynamics for each clone on the different substrates, one can readily observe the analytic difficulties, insomuch as each curve for a specific test is made up of a series of segments.

To obtain a synthetic representation, for each test we used a continuous function, which holds over the entire growth range: goodness of fit of the model with the experimental data has been evaluated by means of the Kolmogorov - Smirnov test.

After preliminary processing, and given the clear sigmoidal shape, a logistic type continuous function was chosen, having the form of Eq. 1, which is characterised by the facts that a biological meaning is assigned to the parameters (Thornley and Johnson, 1990).

$$W_t = \frac{W_0 W_f}{W_0 + \left(W_f - W_0\right) e^{-\mu t}} \qquad \text{(eq. 1)}$$

where:

W_t = biomass at time t, expressed as height [cm];
W_0 = biomass at time t = 0, expressed as height [cm];
W_f = final biomass, expressed as height [cm];
μ = specific growth rate [1/days];
t = time [days]

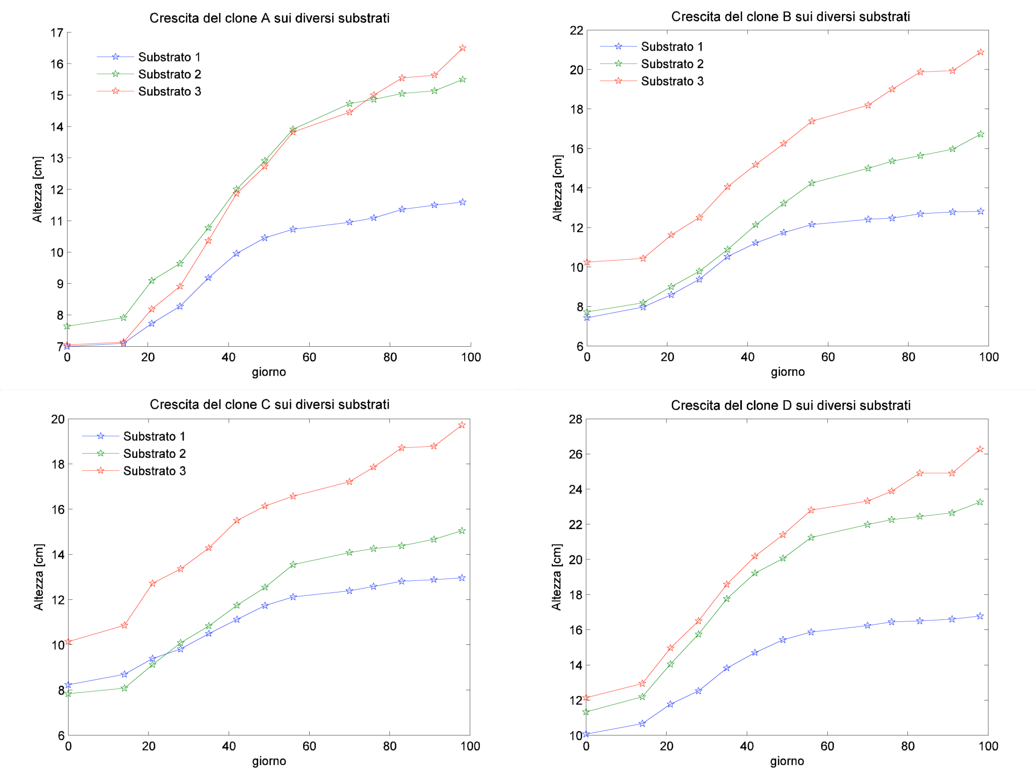


Figure 1. Growth of clones A, B, C and D on the three substrates

Table 4 shows, for each test, the experimental values for W_0, W_f, the resulting growth Δ and the values assigned to μ through a reiterative calibration process.

Table 4. Initial and final height, growth Δ and specific growth rate μ for each test

	Substrate 1				Substrate 2				Substrate 3			
Clone	**W_0**	**W_f**	**Δ**	**μ**	**W_0**	**W_f**	**Δ**	**μ**	**W_0**	**W_f**	**Δ**	**μ**
A	7.00	11.59	4.59	0.109	7.64	15.50	7.86	0.091	7.05	16.50	9.45	0.089
B	7.44	12.82	5.38	0.106	7.72	16.72	9.00	0.083	10.25	20.88	10.63	0.074
C	7.50	12.23	4.73	0.085	7.83	15.04	7.21	0.086	10.14	19.71	9.57	0.063
D	10.06	16.78	6.72	0.101	11.32	23.26	11.94	0.096	12.14	26.26	14.12	0.083
	8.00	13.35	5.35	0.100	8.63	17.63	9.00	0.089	9.89	20.84	10.95	0.077

For the different parameter values used, we found that for all 12 tests the logistic curves fit fairly closely the empirical curve with a significance level μ of 0.05 applying the Kolmogorov- Smirnov test. In the upper part of Graphs x - y, the theoretical logistic curve is compared with the empirical curve, while the lower part shows the (level of) significance of the Kolmogorov-Smirnov test.
Moreover, the differences between the theoretical and empirical curves can be, with a 95% probability, attributed to random factors in 87% of the cases on the sample as a whole (Table 5).

Table 5. Percentage of samples for which the nul hypothesis of the Kolmogorov – Smirnov test is not rejected.

K-S	Substrate 1	Substrate 2	Substrate 3
Clone A	75%	80%	95%
Clone B	80%	91%	86%
Clone C	80%	91%	67%
Clone D	96%	100%	100%

3. DISCUSSION

3.1. Growth **Δ**

As can be seen from Table 4, the growth **Δ**, given by W_f-W_o, appears to depend on the substrate, as the percent increase of organic matter promotes plant growth all-round in all four clones. The growth **Δ** also appears to depend on the clone.
Lastly, the relationship between the initial height W_0 (Table 5) and the growth **Δ** (Table 4), deserve consideration, as a preliminary evaluation shows the latter to be directly proportional to the former.

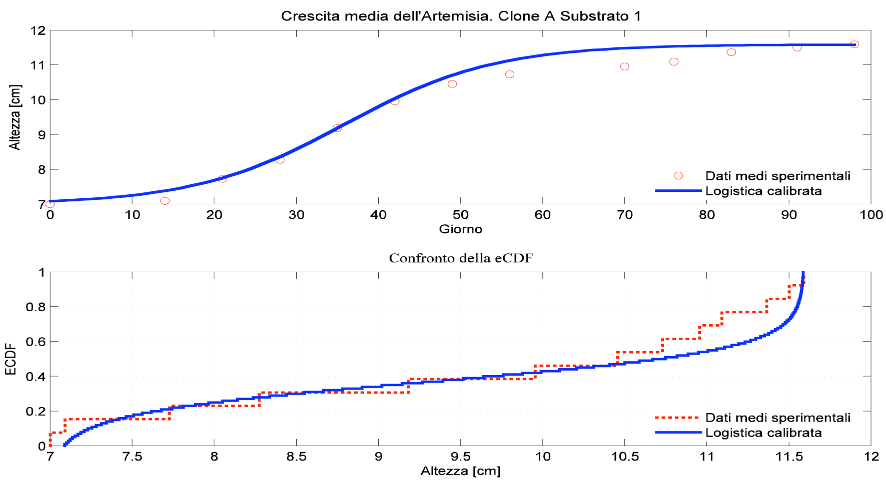


Figure 2. Test 1/A

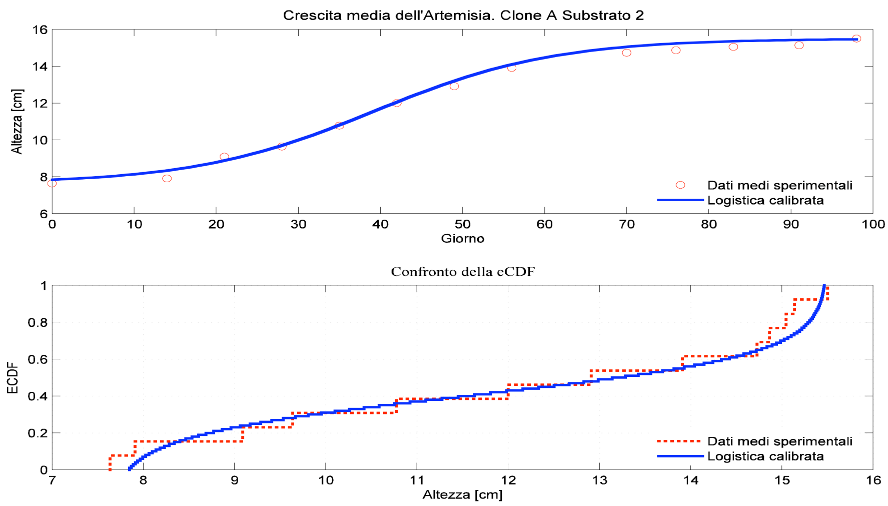


Figure 3 - Test 2/A

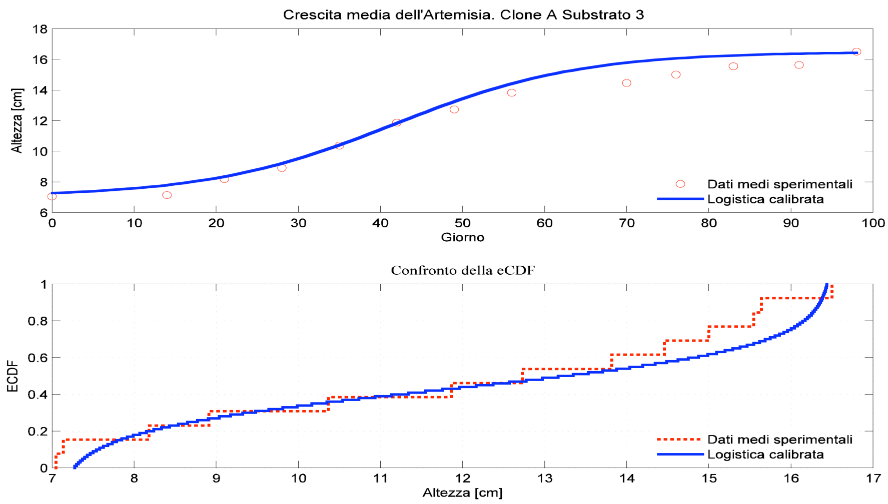


Figure 4 - Test 3/A

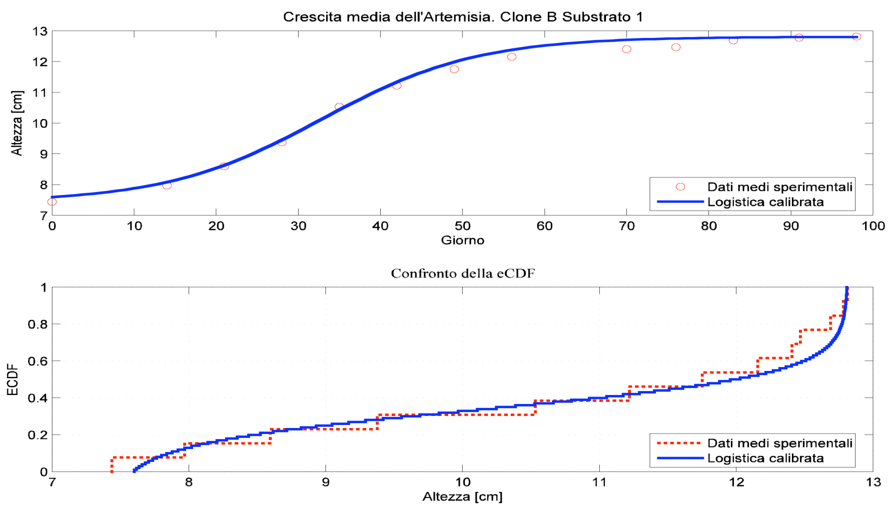


Figure 5 - Test 1/B

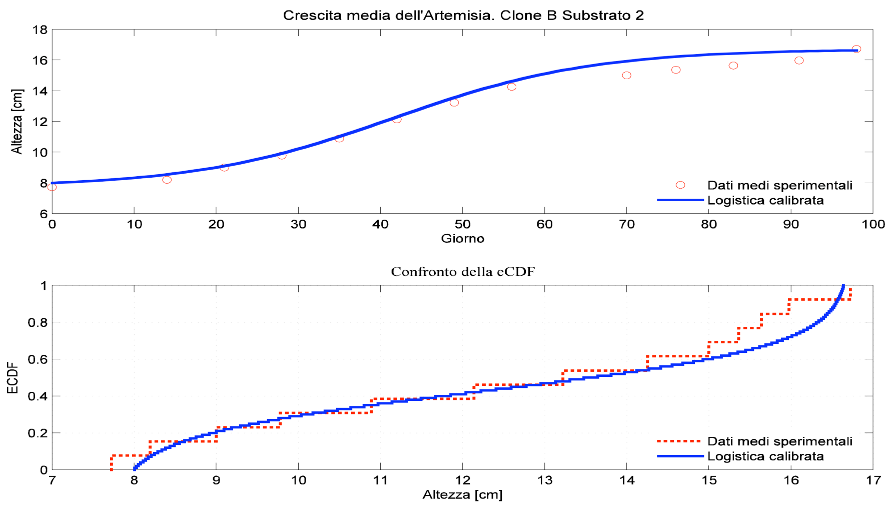


Figure 6 - Test 2/B

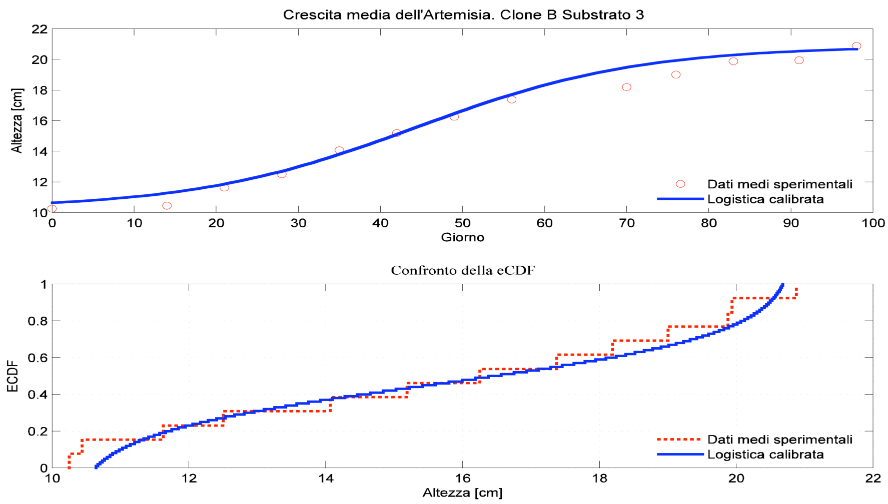


Figure 7 - Test 3/B

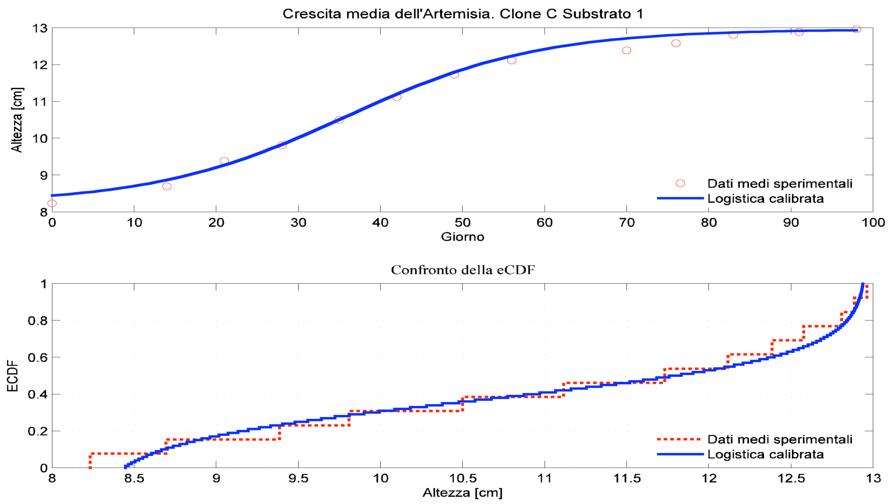


Figure 8 - Test 1/C

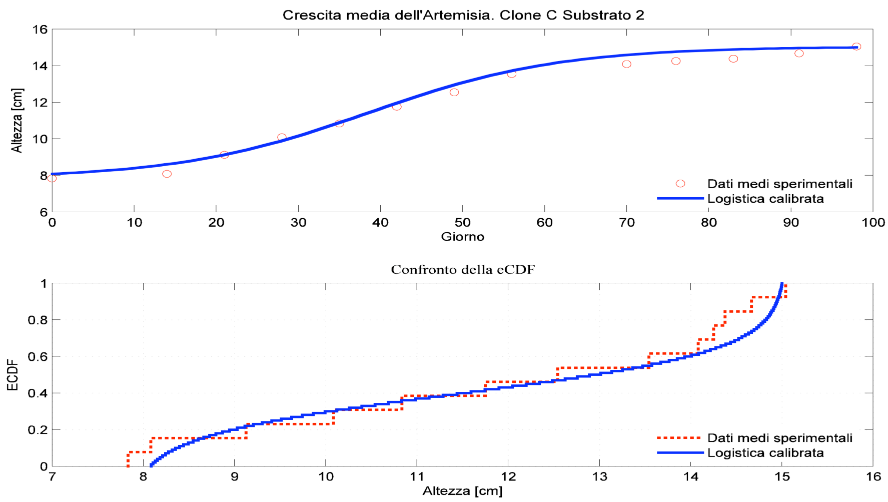


Figure 9 - Test 2/C

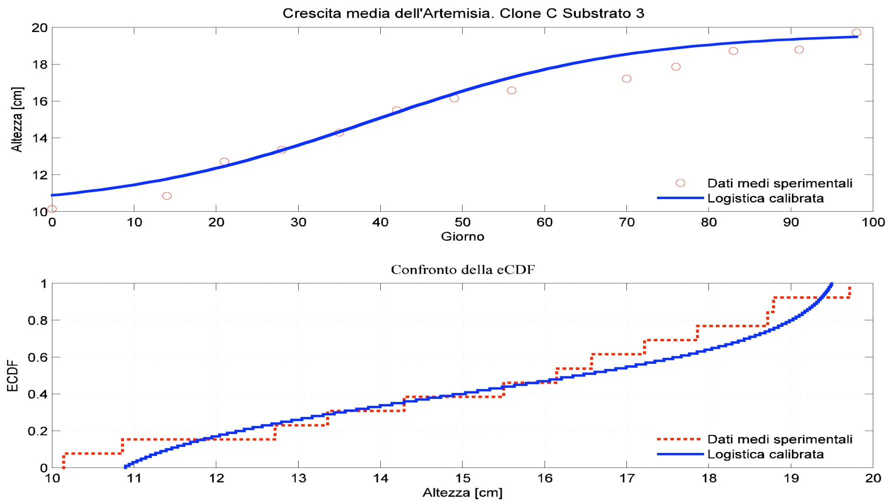


Figure 10 – Test 3/C

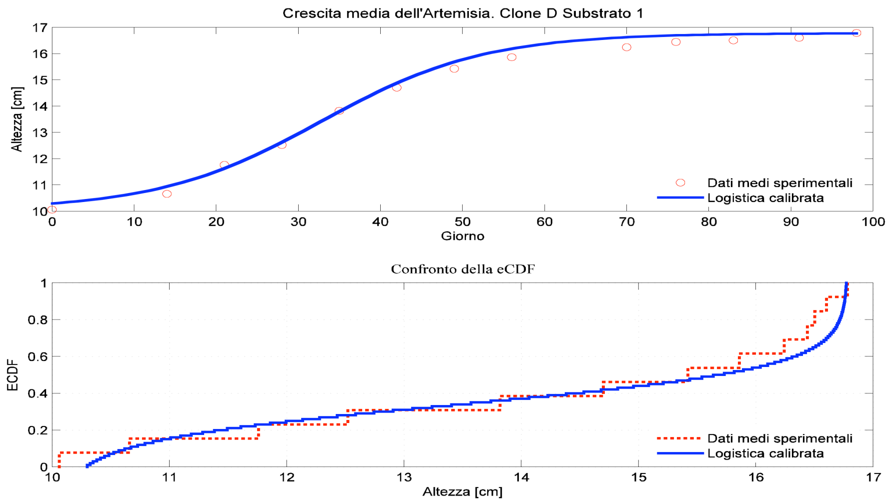


Figure 11 - Test 1/D

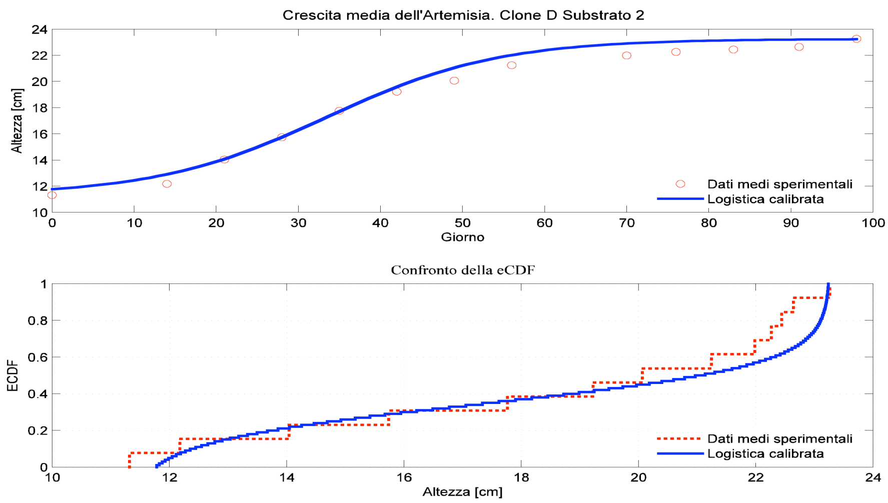


Figure 12 - Test 2/D

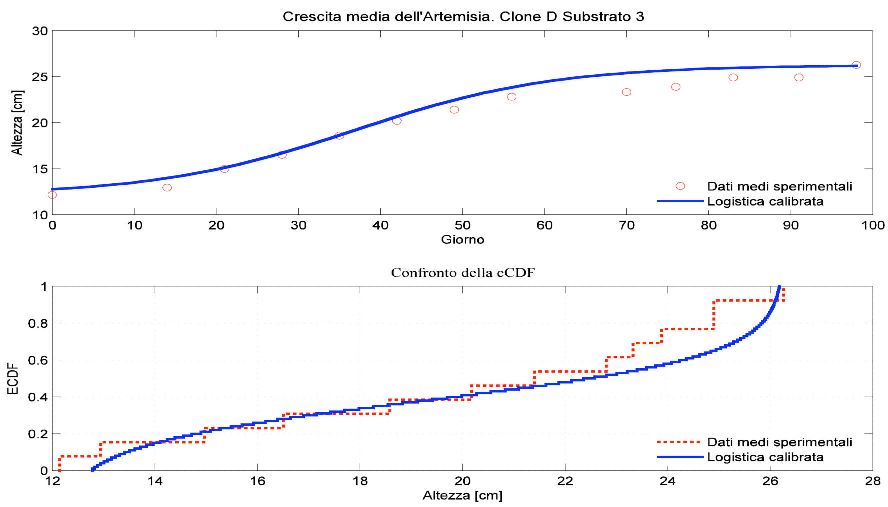


Figure 13 - Test 3/D

3.2. Hypotheses on the parameter μ

The parameter μ has a biological meaning and affects what Thornley and Johnson call the "growth machinery" (Thornley & Johnson, 1990). In this study, the specific growth rate μ is determined for the purpose of exploring the influence, be it positive or negative, of the substrate and of the genetic factor on growth, by varying the values taken by the parameter μ.
A cursory evaluation appears to show a correlation between the substrate and the value taken by μ, in the sense that the latter decreases, on average, passing from a value of 0.100 in substrate 1 to 0.089 in substrate 2 to 0.077 in substrate 3.
Additionally, the trend for each single clone matches the average behaviour, with the exception of clone C for which μ is 0.85 in substrate 1 and 0.86 in substrate 2, showing the reverse trend, be it with a minor deviation.
This first observation and comparison of the values of μ with growth Δ (Table 4), suggest that greater plant height (more organic matter) coincides with a lower specific growth rate μ. As μ, in the case at hand, is expressed by the rate at which the plant grows (graphically the slope of the

curve increases in that segment where the curve bends), this translates into in initial, more rapid increase in growth for the plant that grows however to lower heights.
By contrast, preliminary analysis suggests that no correlation exists between the different clones and μ, i.e. no relationship has been detected between the genetic factor and μ.
For each group, the theoretical curves for each test have been discretised and the average values taken for all the measured values. In this way we found the logistic curve representing the "substrate" group, and by so doing eliminated from the analysis the genetic factor due to the different clones.
The next step consisted is the pairwise comparison of the three curves to determine, by means of the Kolmogorov – Smirnov test, whether the differences could be attributed to systematic factors, in this case the substrate.
Thus we have tests 1 – 2, 1 – 3 and 2 – 3. For the tests 1 – 2 and 1 – 3, the K-S null hypothesis is rejected, while only for the test 2 – 3 can the differences between the theoretical and experimental curves be attributed to random factors, with a 95% probability (Table 6).
This means that the systematic factor may coincide with the substrate, even though, as can be seen from the graphs (Figure 14, Figure 15 and Figure 16), the curves have different initial values of W_0. So we translated one of the two curves such that they started with the same initial value and repeated the series of tests. Had the condition of systematicity not been satisfied, this would have suggested that the systematic factor resided in the initial Δ.
However, the analysis simply confirmed the previous tests, thus no more precise considerations can be drawn on the initial value of the height W_0.
The same reasoning was applied to evacuate the genetic factor and any influence it may have on the parameter μ Four groups of tests were conducted:

Clone A: 1/A , 2/A, 3/A;
Clone B: 1/B , 2/B, 3/B;
Clone C: 1/C, 2/C, 3/C;
Clone D: 1/D , 2/D, 3/D;

A pairwise comparison of the theoretical curves was performed, making a total of six: A – B, B – C, C – D, A – C, B – D, A – D.
Similarly to above, translating one of the two curves so that they both had the same initial value of W_0 did not alter the results of the Kolmogorov – Smirnov test.
The results of our tests revealed that only in the comparison between the clones A, B, C and D, are the differences attributable, with a 95% probability, to systematic factors (Table 7). In addition to the possibility that differences in the value of W_0 give rise to statistically significant differences (W_0 are greater for clone D), we can assume that a certain genetic component may influence the value of μ, also considering that the growth Δ were higher for clone D.

Table 6. Kolmogorov - Smirnov test on the three comparisons for the substrate. $H_0 = 1$ hypothesis rejected

	H_0	p - Value	D_n
Test 1 - 2	1	0.028	0.615
Test 1 - 3	1	0.002	0.692
Test 2 - 3	0	0.087	0.461

Table 7. Results of Kolmogorov test on comparison between clones

	H_0	P - value	D_n
Test A – B	0	0.087	0.461
Test B – C	0	0.226	0.385
Test C – D	1	0.002	0.692
Test A – C	0	0.226	0.385
Test B – D	1	0.008	0.615
Test A – D	1	0.0003	0.769

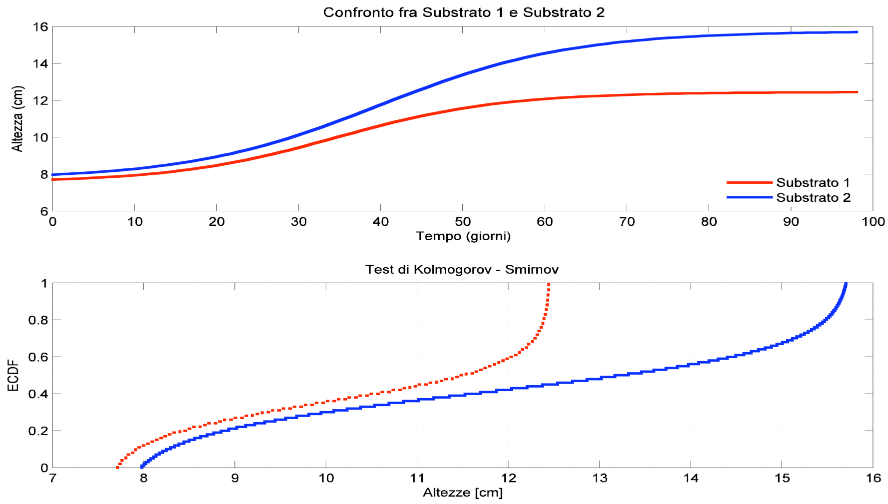


Figure 14 – Comparison of theoretical curves for substrates 1 and 2, with Kolmogorov-Smirnov test

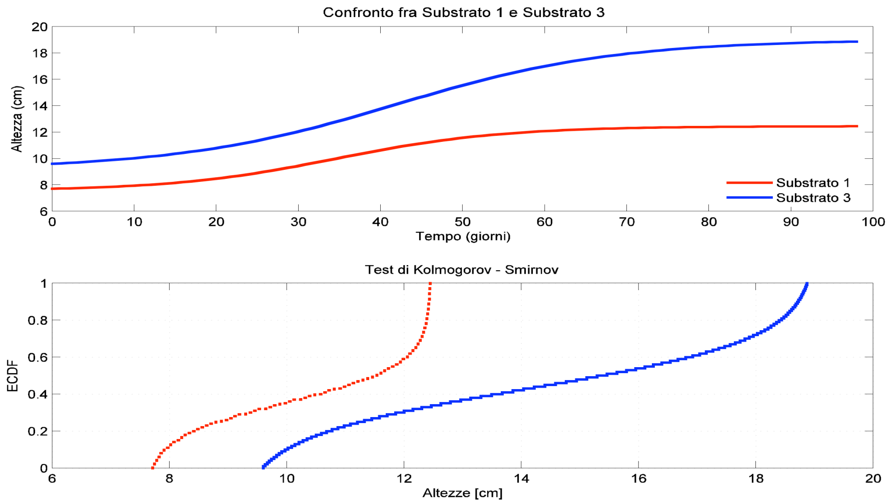


Figure 15 - Comparison of theoretical curves for substrates 1 and 3, with Kolmogorov- Smirnov test

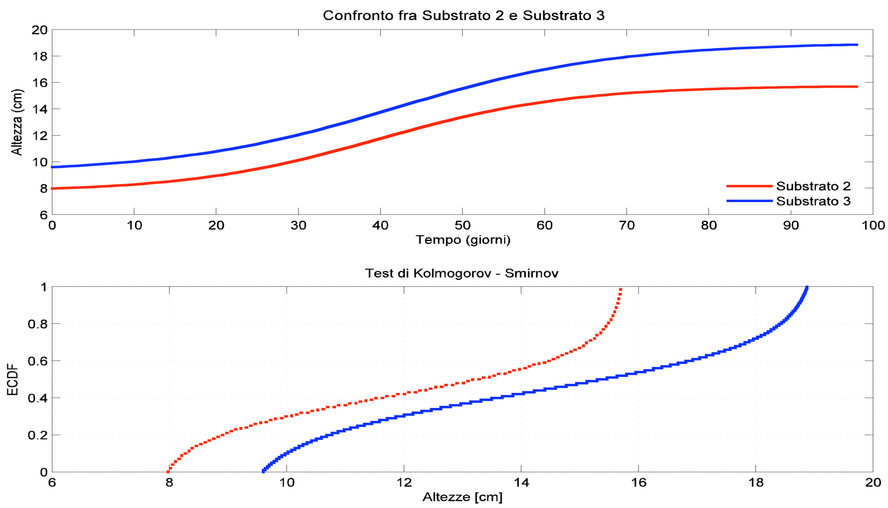


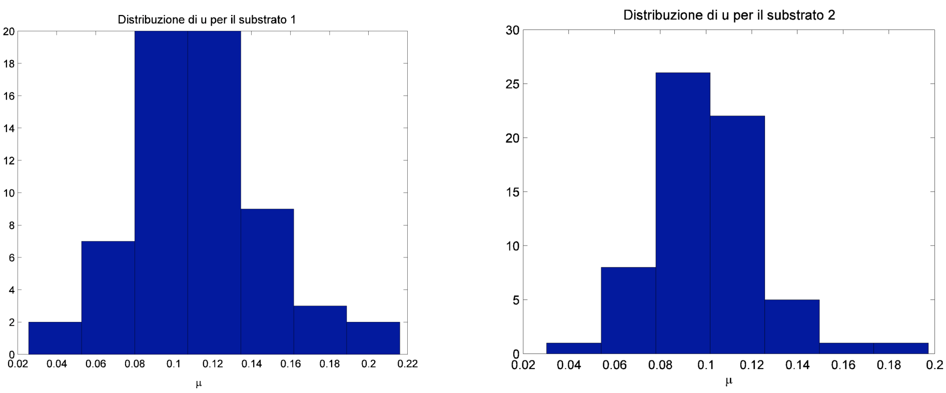
Figure 16 - Comparison of theoretical curves for substrates 2 and 3, with Kolmogorov – Smirnov test

3.3. Distribution of specific growth rate μ.

The parameter μ relative for a clonal population or for a certain substrate (or both) is normally distributed if the organic matter content is sufficiently high and thus the phenomenon is controllable.

By reducing the proportion of slops with respect to the inert matter in the artificial substrate composition, the distribution of the parameter μ passes from normal to chaotic once a certain threshold is reached. Thus the phenomenon can no longer be described by means of the logistic curves in this specific case. The aim is to determine the minimum percentage in relation to the percent proportion of distilling slops.

As only a small number of data (less than 10) were available for tests 3/B and 3/C, it was considered more appropriate to determine the distribution of μ as a function of the substrate and of the type of clone rather than as a function of each single test. The Lilliefors test ($\alpha = 0.05$) was then used to determine whether the distribution was normal or otherwise. The test was performed first directly on the distribution of μ and then also on the log transform to compensate for skew to the right.



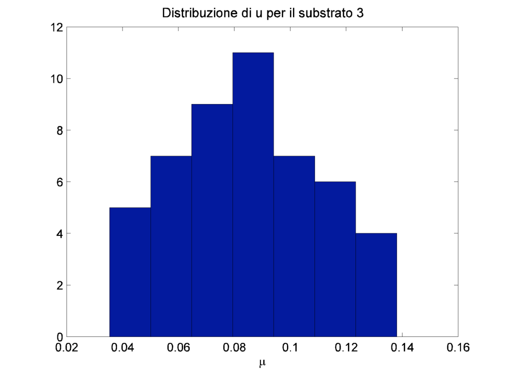


Figure 17 - Distribution of μ for substrate

Table Errore. Nel documento non esiste testo dello stile specificato.**8. Statistical data on the distribution of μ for substrate**

	μ_m	σ	γ_1	γ_2
Substrate 1	0.1105	0.0352	0.5132	3.8798
Substrate 2	0.0999	0.0248	0.6432	6.1003
Substrate 3	0.0862	0.0285	0.3254	2.5400

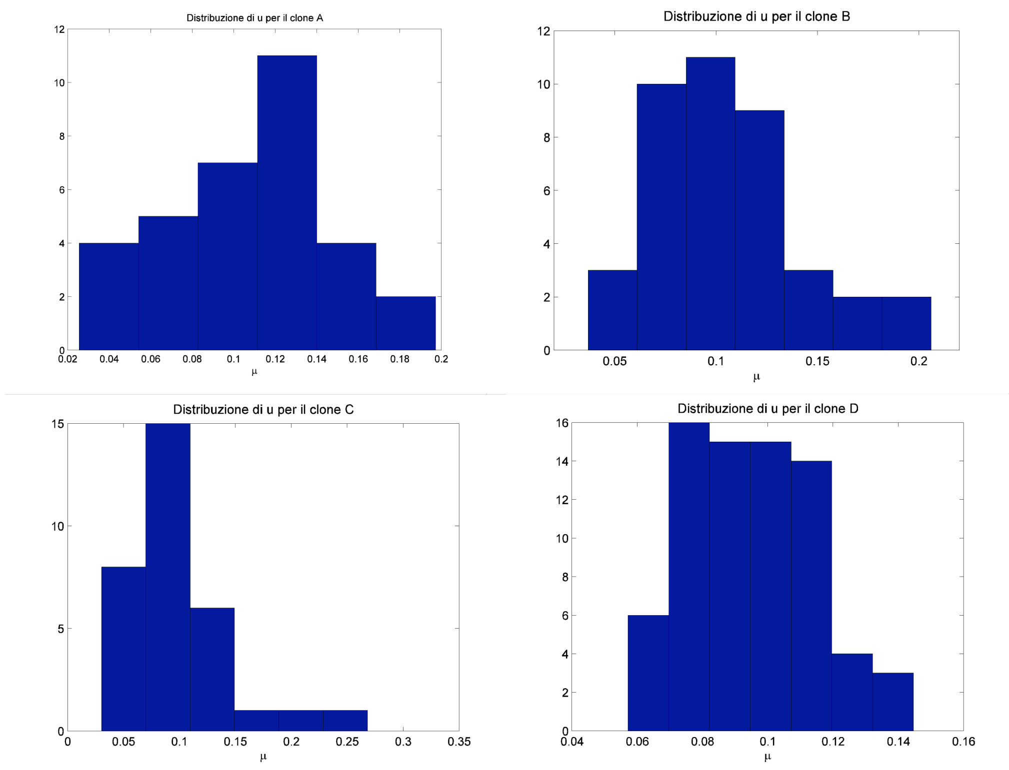


Figure 18 - Distribution of μ for clone

Tabella 9. Statistical data on the distribution of μ for clone

	μ_m	σ	γ_1	γ_2
Clone A	0.1097	0.0387	- 0.1702	2.8509
Clone B	0.1053	0.0370	0.8587	3.7889
Clone C	0.1014	0.0475	1.5183	6.3761
Clone D	0.0960	0.0195	0.3321	2.7177

Considering the distributions of μ for the substrate, using the Lilliefors test the null hypothesis is rejected for substrate 1, but not for substrates 2 and 3. Performing the test on the log transform however, the null hypothesis (in other words μ has a normal distribution) is never rejected.
On the other hand considering the clones, μ is normally distributed, with a 95% probability given that the null hypothesis is never rejected. Performing the test on the log transform only for clone A is the hypothesis rejected.

CONCLUSIONS

Analysis of the experimental data obtained from previous tests on the growth of four cloned populations A, B, C and D of the *Artemisia arborescens* L. species on three different artificial substrates, obtained by mixing different proportions of mineral matter and distilling slops, revealed several aspects of the phenomenon and a number of hypotheses have been advanced that warrant further investigation.
The findings of the experimental work confirm that *Artemisia arborescens* L, grows well on contaminated substrates improved with the additino of suitable quantities of organic matter (distilling slops in the specific case).
In particular, it has been demonstrated that plant growth, measured as growth Δ (difference between initial and final height) is influenced by both the substrate (organic matter content) and the genetic make up (clone D grows to greater heights than the others, followed by clones B, A and C). For clones A and C the influence of the genetic makeup on growth Δ has been shown (7.30 cm for A against 7.17 cm for C) also for clone A with lower initial heights for (7.23 cm against 8.49 cm del clone C).
Application of the non-parametric Kolmogorov – Smirnov test ($\alpha = 0.05$) confirmed that the logistic equation has a statistically significant goodness of fit with the experimental data: for 87% of the sample and for all 12 tests the null hypothesis (the data are logistically distributed) of the Kolmogorov - Smirnov test is not rejected. This shows that the differences between the theoretical logistic curves and the empirical curves can be attributed to random factors.
In particular, the fit between theoretical and empirical curves improves from substrate 1 to 2 and from 2 to 3, i.e. increasing the organic matter content, as this ensures more regular plant growth. This is not however true of tests 3/B and 3/C but this can be explained by the small number of data used.
The specific growth rate parameter was determined in order to explore the influence of μ on the substrate and genetic makeup from a qualitative point of view. The mean value of μ is 0.089.
It has been shown that the specific growth rate decreases with increasing organic matter content (distilling slopes), 0.1 for substrate 1, 0.089 for substrate 2 and 0.077 for substrate 3. Thus growth diminishes for those plants exhibiting greater growth Δ, because of the need for plants to grow as harmoniously as possible in order to develop better.
The influence of genetic makeup on μ is less evident: it only has statistical significance for clone D.
Lastly, μ is normally distributed and thus it can be claimed that up to an organic matter content of 10%, plant growth can be described by a logistic equation. Thus organic matter can be further reduced until a threshold can be established below which the distribution of μ becomes random and the phenomenon can no longer be well described by a logistic type curve.

REFERENCES

Thornley J., & Johnson I., (1990) - *Plant and Crop Modelling*. Clarendon Press, Oxford