Estimation of the dry leaf biomass of *Lippia graveolens* Kunth in southeastern *Coahuila*

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Abstract

Oregano is a non-timber forest resource of commercial importance, considered as a culinary and aromatic species that is used in the semi-arid areas of *Coahuila*, with a yield of more than 700 t per year and representing a productive option for the rural sector. With the purpose of quantifying the resource and helping regulate its use, the allometric relationships of individuals of oregano collected in 20 natural populations distributed in the municipalities of *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe*, *Coahuila* were determined, so as to select a model that estimates the dry leaf biomass (*DLF*) of the plant. Based on a destructive sampling, 706 plants were analyzed; their total height (*TH*), the larger diameter (*LD*) and smaller diameter (*SD*) of the bush, the mean diameter (*MD*) of the crown and the dry leaf biomass (*DLB*) were calculated. The variables most related to the *DLB* were selected with Pearson's correlation test and utilized to adjust 10 regression models according to the PROC MODEL procedure. The model of Schumacher-Hall *DLB = 0.00599(MD)$^{1.935454}$(TH)$^{0.256803}$* was used for recording R^2_{adj} values above (0.80), as well as the lowest value for the root mean square error (RMSE, 0.304), considering the significance of its parameters *(p≤ 0.0001)*; based on this, a double-entry table estimating the DLB of the plants was developed.

Key words: Allometry, dry leaf, forest management, non-timber, oregano, *Coahuila*.

Introduction

Lippia graveolens Kunth (synonym: *Lippia berlandieri* Schauer) is a wild, aromatic plant commonly known as Mexican oregano or scented matgrass, which is distributed in at least 24 entities of the arid and semiarid regions of Mexico (Villavicencio *et al.*, 2007). Its largest production for commercial purposes comes from natural populations; it is the species with the most widespread distribution in Mexico (Rueda, 2015; Trópicos, 2016).

In the northern region of the country, the main exploitation areas, with the highest production of oregano leaves, are located in the states of *Chihuahua*, *Coahuila*, *Durango* and *Tamaulipas*; these add up to 50 % of the authorizations for the collection of the plant and are followed by the states of *Zacatecas*, *Querétaro*, *Hidalgo* and South *Baja California* (Huerta, 2002; Conafor, 2017).

Oregano is a non-timber forest resource of which 6 500 t per year are harvested; 90 % of these are destined for the export market. It is known commercially as Mexican oregano (INFOAGRO, 2006; Villavicencio *et al.*, 2007) and it has a great potential in the national and international agri-food chain when its uniform production in terms of both quality and quantity is ensured (Huerta, 2002, Huerta, 2005). The main product derived from the leaf of this plant is the essential oil, which is used in the food, liquor, soda, pharmaceutical and cosmetic industries. Oregano belongs to the group of spices and cooking herbs; however, its uses are not limited to these purposes: it is also utilized as an additive in other products (FAO and OMS, 2017). The main market for its essential oil is the United States of America, followed by Italy and Japan (Gaby *et al*., 2003; Conafor, 2009).

In the semi-arid region of *Coahuila*, *L. graveolens* is exploited in eight municipalities; the largest production is obtained from *Parras de la Fuente*, *General Cepeda* and *Ramos Arizpe* (INAFED, 2005; Villavicencio *et al.*, 2010).

L. graveolens is a shrub; it changes in size and shape due to the adaptation of the species to the environmental conditions, which generates differences in its development, which are reflected in its allometric variability, its height being directly proportional to its diameter (Niklas, 1995). Based on this relationship, it is possible to carry out dimensional analyses whereby the predictive models can be adjusted in order to generate individual biomass yield tables for quantifying the production and promoting the rational, sustainable exploitation of the species.

The dimensional analysis is a reliable estimation technique for calculating the biomass and volume of the plants based on easily measured variables (Porté *et al.*, 2000), particularly in the case of tree species of temperate forests (Návar, 2010), tropical taxa (Wiant and Charton, 1984; Gaillard *et al.*, 2002; Barrios *et al.*, 2014) and, less frequently, shrubs (Laamouri *et al.*, 2002; Guillen *et al*., 2007).

In species of arid zones, the dimensional analysis is carried out in order to calculate the leaf biomass of *Larrea tridentata* (Sessé & Moc. ex DC.) Coville (*gobernadora*) (Ludwig *et al.*, 1975), mesquite (*Prosopis glandulosa* Torr.*)* (Whisenant and Burzlaff, 1978; Méndez *et al.*, 2012), and acacia (*Acacia pennatula* (Schltdl. & Cham.) Benth) (López-Merlín *et al.*, 2003), and even in the giant Spanish dagger (*Yucca carnerosana* Trel.)McKelvey) (Villavicencio and Franco, 1992) and lechuguilla (*Agave lechuguilla* Torr.) (Berlanga *et al.*, 1992); determine the weight of the bud and proportion of fiber in sotol (*Dasylirion cedrosanum* Trel.), and estimate the weight of the stem or "core" (Cano *et al.*, 2006) in cortadillo (*Nolina cespitifera* Trel.) (Saenz and Castillo, 1992) and in fodder shrubs such as *Atriplex canescens* (Pursh) Nutt, in order to calculate the dry weight of the aerial biomass (Thomson *et al.*, 1998).

The sustainable use of natural populations of oregano requires reliable estimates of the production of dry leaves of the plants under management. Therefore, the objectives of this study were: a) to determine the allometric relationships in

oregano plants for 20 natural populations distributed in the municipalities of *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe*, *Coahuila*; b) to select the predictive model with the best fit to estimate the dry biomass, and (c) to generate a dry leaf biomass production table of standing oregano shrubs.

Materials and Methods

Study area

The research was conducted in natural populations of oregano distributed in *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe* municipalities, located between the coordinates 25°22'41" - 25°26'27 N and 100°57'2" - 102°11'10" W, with an altitudinal range of 1 000 to 1 400 m. The predominant soil types are Lithosol, Xerosol and (calcic and haplic) Yermosol with a medium texture and without salinity issues (Inegi, 2005). The climate of the region, according to Köeppen's classification modified by García (2004) and Inegi (2005) is of the BS_1hw (semi-arid and semi-warm) and BS_0hw (very arid semi-warm) types, with a mean temperature of 18 to 20 °C, and extreme values from -4 to 45 °C, with an annual precipitation of 125 to 400 mm.

Oregano is a shrubby plant with annual sprouts, associated to the vegetation consisting of rosetophilous scrub with agave of Salm (*Agave salmiana* Otto ex Salm-Dyck), *lechuguilla* (*Agave lechuguilla* Torr.), *xoconostle* (*Opuntia imbricata* (Haw.) DC.), *candelilla* (*Euphorbia antisyphilitica* Zucc.), *sotol* (*Dasylirion cedrosanum* Trel.), Prickly Pear Cactus (*Opuntia* spp.), *espadín* (*Agave striata* Zucc.), and *maguey* (*Agave* spp.).

In the microphyllic shrub, with *hojasen (Flourencia cernua* DC*.),* skeletonleaf goldeneye *(Viguiera stenoloba* S.F. Blake), *mariola* (*Parthenium incanum* Kunth), *gobernadora*, *tasajillo* (*Opuntia leptocaulis* DC*.*), *ocotillo* or coachwhip (*Fouquieria*

splendens Engelm.*)*, *guayule* (*Parthenium argentatum* A. Gray), giant Spanish dagger (*Yucca carnerosana* (Trel.) McKelvey), *coyotillo* (*Karwinskia humboldtiana* (Schult.) Zucc.*),* leatherstem or *sangre de drago* (*Jatropha dioica* Sessé), woody crinklemat (*Tiquilia canescens* (A. DC.) A.T. Richardson) and *Polieria angustifolia* (Engelm.) A. Gray.

In both types of vegetation, oregano is associated with different species of cacti and with the stratum of taxa, such as wicker (*Chilopsis linearis* (Cav.) Sweet), *mesquite* (*Prosopis sp.*) and *huisache* (*Acacia farnesiana* (L.) Willd. and *Acacia constricta* Benth.) (Berlanga *et al*., 2005).

Data collection and sampling design

The sample was composed of 706 individuals distributed in 20 populations located in three municipalities of southeastern *Coahuila*. The sampling was carried out during the harvesting period of the plant (July to October), and each population was georeferenced for its location (Table 1 and Figure 1).

Table 1. Number of sampled oregano populations and plants in *General Cepeda, Parras de la Fuente* and *Ramos Arizpe* municipalities, corresponding to the oregano corridor in *Coahuila*.

Municipality		Locality	Number of populations	Individuals sampled
General Cepeda	1	*Independencia*	11	401
	2	*Independencia 2*		
	3	*Narigua*		
	4	*La Parrita*		
	5	*Porvenir de Jalpa*		
	6	*Duraznillo*		
	7	*Rincón Colorado*		
	8	*Parrita*		
	9	*La Rosa*		
	10	*Porvenir de Tacubaya*		
	11	*Agua de Mula*		
Parras de la Fuente	1	*San Isidro*	4	118
	2	*San Rafael*		
	3	*Boquillas del Refugio*		
	4	*Cuatro de Marzo*		
Ramos Arizpe	1	*Sacrificio*	5	187
	2	*Paloma*		
	3	*Plan Guadalupe*		
	4	*Las Coloradas*		
	5	*Sauceda*		
Total			20	706
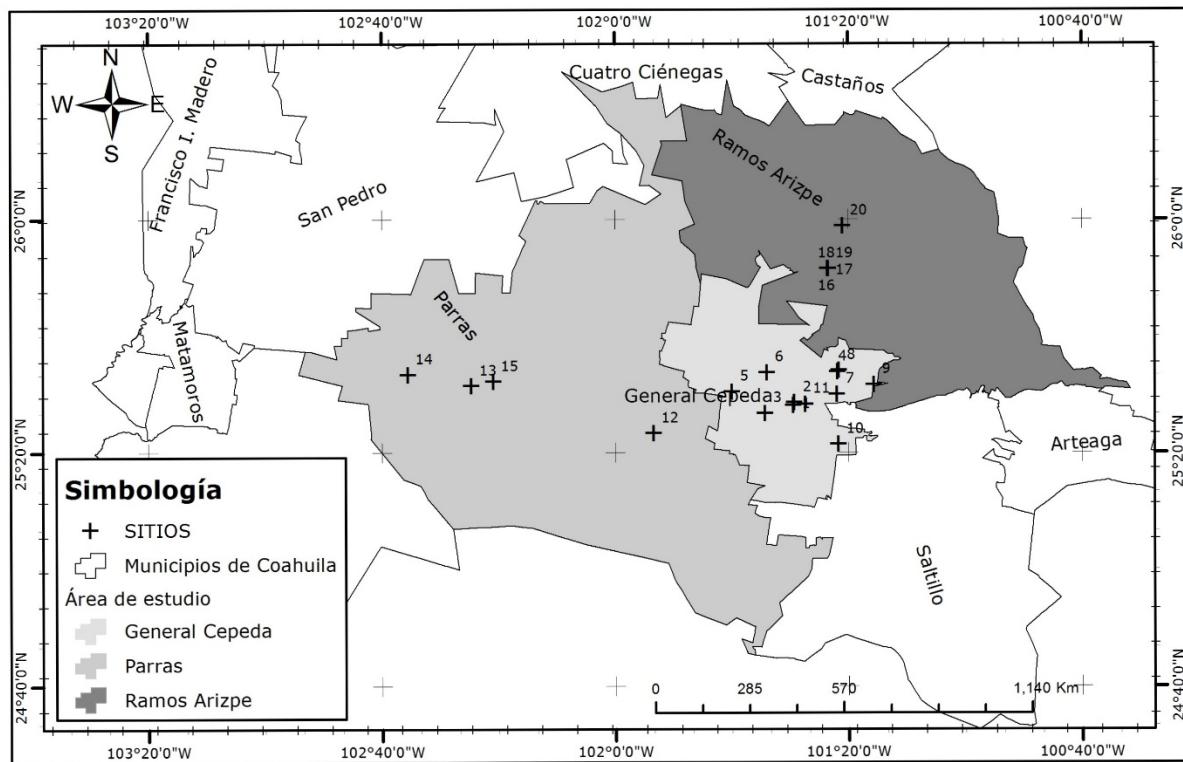
Figure 1. Geographical distribution of the sampling localities in *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe* municipalities, *Coahuila*.

Independent Variables

The total height (*TH* cm), the larger diameter (*LD*, cm) and smaller diameter (*SD*, cm) of the shrub cover were measured in each sampled individual with a Truper™ tape model 21601 (Figure 2 b, c). In order to take into account the variability of the growth of the species, all the categories of height and cover of the standing shrubs present in the populations were included. The *TH* of the plant was measured from the base of the soil to the tip of the highest branches (Figure 2a), the *LD* and *SD* were measured taking into account the shrub cover (Figure 2b and 2c). The mean diameter (*MD*, cm) of the cover was estimated based on the larger and smaller diameters.

Figure 2. Measurement of a) height (*TH*), b) larger diameter (*LD*) and (c) smaller diameter (*SD*) of *Lippia graveolens* Kunth shrub.

Dependent Variable

The dry leaf biomass (*DLB*, gr) was calculated based on a destructive sampling of the assessed individuals; for this purpose, the whole aerial part of the plants (stems and leaves) was harvested and stored in paper bags. The samples were then dehydrated *in situ* at room temperature for five days —a drying method used by the producer. The dry biomass was subsequently separated by components: stems and leaves. The weight of dry leaves per sample was determined on a *Schientech* analytical balance with an accuracy of 0.001 g. The *DLB* per plant —*i.e.* the usable component with commercial importance— was thus estimated.

Statistical Analysis

The set of data on the *DLB* and the allometric variables *TH, LD* and *SD* were analyzed, first, through a Pearson's correlation test in order to select those variables most related to the *DLB* that were used to adjust linear and non-linear regression models (SAS, 2015) (Table 2).

Table 2. Models adjusted to estimate the dry leaf biomass in natural populations of *Lippia graveolens* Kunth, in *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe* municipalities, *Coahuila*.

Num.	Model	Structure
1	Allometric	$DLB = B_0\,(MDTH)^{B_1}$
2	Constant morphic coefficient	$DLB = B_1\,(MD^2TH)$
3	Australian model	$DLB = B_0 + B_1 MD^2 + B_2 TH + B_3 MD^2 TH$
4	Combined Linear Variable	$DLB = B_0 + B_1(MD^2TH)$
5	Spurr	$DLB = B_1(MD^2TH)^{B_2}$
6	Schumacher-Hall	$DLB = B_0(MD)^{B_1}(TH)^{B_2}$
7	Schumacher	$DLB = B_0 e^{(-B_1/MD)}$
8	Potency	$DLB = B_0(MD)^{B_1}$
9	Takata	$DLB = \dfrac{MD^2TH}{(B_0 + B_1 MD)}$
10	Thornber	$DLB = B_0\left(\dfrac{TH}{MD}\right)(MD^2TH)$

MD = Mean diameter of the cover (cm); TH = Total height (cm); B_n = Model parameters; e = Exponential expression.

The database used for the regression analysis was purged through the detection of Outlier with *the r-influence* of the SAS 9.4 software (SAS, 2015), in order to eliminate potential errors in the data base that might affect the statistical regression. The selected model was the one that exhibited the highest values for the adjusted determination coefficient (R^2_{adj}) and the lowest value for the root mean square error (RMSE), in addition to the significance of its parameters (P ≤ 0.001). The regression assumptions were verified using the Shapiro-Wilk test for normality; the White test, to detect heteroskedasticity, in which, due to the nature of the data, a correction was assumed and applied by weighting the form residuals, $1/_{MD*TH}$, whereby this problem was eliminated; finally, the Durbin-Watson statistic test was carried out in order to test the co-linearity between variables. The regression models were adjusted with the the PROC MODEL procedure to generate consistent estimators (SAS, 2015).

Results and Discussion

Equation for estimating DLB

The correlation test determined that the variables *MD* and *TH*, and the interaction between them, have a significant relationship with the *DLB* (p< 0.001), of 0.82, 0.53 and 0.83, respectively, with the rest of the evaluated variables, and therefore they were used as the basis for the adjustment of the models. The statistical adjustment was similar for all models, particularly models 3 and the 6, which exhibited higher R^2_{adj} values and lower RMSEs. However, these had heteroskedasticity issues and therefore were corrected; thus, the correlation was obtained only for model 6, and for the adjustment values of R^2_{adj} (0.81) and of the RMSE (21.5256), which was selected to estimate the *DLB* of oregano.

The structure of the chosen model corresponds to that of Schumacher-Hall, which is sigmoidal (Table 3); furthermore, the model uses the *MD* and *TH* that characterize the shape of the oregano shrub and based on which a double-entry table was built enabling the primary producers to estimate the *DLB*s.

Table 3. Adjustment statistics and values of the parameters of the models analyzed in order to estimate the dry leaf biomass of *Lippia graveolens* Kunth.

Num.	SSE	RSME	R^2_{adj}	B	Parameter	Pr>|t|
1	455 776	25.8894	0.7253	B_0	0.000306	<0.0001
				B_1	1.424362	<0.0001
2	349 222	22.6452	0.7898	B_1	0.000167	<0.0001
3	306 415	21.2589	0.8148	B_0	11.10985	0.0913
				B_1	0.006091	<0.0001
				B_2	-0.22978	0.0047
				B_3	0.000117	<0.0001
4	338 318	22.3053	0.7961	B_0	5.405543	0.0007
				B_1	0.000158	<0.0001
5	345 907	22.5541	0.7915	B_1	0.000344	<0.0001
				B_2	0.946499	<0.0001
6	314 615	21.5256	0.8101	B_0	0.005990	0.0005
				B_1	1.935454	<0.0001
				B_2	0.256803	0.0009
7	381 556	23.6878	0.7700	B_0	1204.998	<0.0001
				B_1	-197.942	<0.0001
8	318 768	21.6513	0.8079	B_0	0.001766	<0.0001

				B_1	2.484405	<0.0001
9	348 731	22.646	0.7898	B_1	6309.38	<0.0001
				B_2	-3.69086	0.1018
10	318 285	21.6349	0.8081	B_0	0.000173	<0.0001
				B_1	-0.44604	<0.0001

SSE = Sum of squares of the error; RMSE = Root mean square error; R^2_{adj} = Determination coefficient adjusted by the number of parameters; B = parameter of the model; Pr>|t| = Significance (p<0.005).

The Schumacher-Hall model is applicable for these conditions and study sites; this type of model has been used to predict the timber volume of temperate species, as well as their total biomass and components (Velasco *et al.*, 2007; Ramos-Uvilla *et al.*, 2014), while the potency model has been used in *Acacia pennatula* and *Guazuma ulmiflora* to predict the forage biomass and fuelwood production, considering the mean basal diameter as a variable (López-Merlín *et al.*, 2003).

The results show that the composition of the shrub exerts an influence on the allometric relationships of the plant; the *TH* and *MD* are related variables that show a phenotypic plasticity in response to environmental heterogeneity with morphological and physiological adjustments (Camargo *et al.*, 2008). The same variables were also considered in *Lysioma divaricatum* (Jacq.) J.F.Macbr. (Breceda and Ortiz, 2005) and in *Cercidium floridum* Benth. ex A.Gray (forage plant) in order to predict the forage production (Guillen *et al.*, 2007); these variables are therefore easy to measure in field and can help evaluate the populations of oregano for exploitation.

The Schumacher-Hall model for estimating the *DLB* was structured as follows:

$$DLB = 0.00599(MD)^{1.935454}(TH)^{0.256803}$$

The *MD* and *TH* are significantly correlated with the dry leaf biomass of oregano; therefore, their estimates are reliable and can be used by the Providers of Professional Services (PPSs) to estimate the dry leaf biomass in the region of the three municipalities considered. Graphically, there is a good dispersion of the estimated data against the observed data, as well as a good fit (Figure 3).
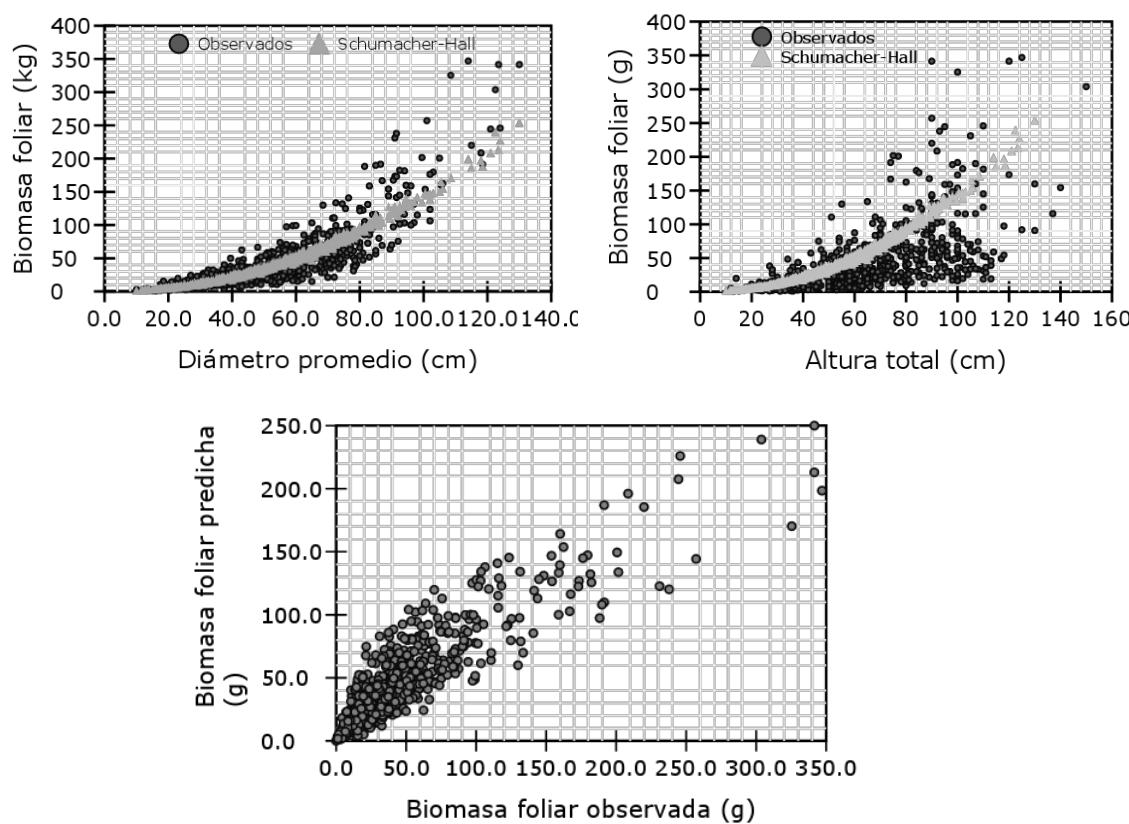
Biomasa foliar = Dry leaf biomass; *Diámetro promedio* = Mean diameter; *Biomasa foliar predicha* = Dry leaf biomass predicted; *Biomasa foliar observada* = Dry leaf biomass observed

Figure 3. Dry leaf biomass (*DLB*) values observed and predicted for *Lippia graveolens* Kunth with the Schumacher-Hall Model.

Table of production (g) of dry leaves of *Lippia graveolens* Kunth

The Schumacher-Hall model was used to develop a double-entry table for estimating the *DLB* of oregano in three municipalities of *Coahuila*. The measurement variables that serve as input are the height and mean diameter of the shrub's cover, both of which are expressed in centimeters. Based on these variables, it is possible to determine the weight in grams of the dry leaves in the standing shrubs without having to cut them (Table 4).

Table 4. Production (g) of dry *Lippia graveolens* Kunth leaves in terms of the diameter of the cover and the height of the shrubs in natural stands located in *Parras de la Fuente*, *General Cepeda* and *Ramos Arizpe* municipalities, *Coahuila*.

MD (cm)	Height (cm)													
	10	15	20	25	30	35	40	45	50	55	60	65	70	75
5	0.24	0.27												
10	0.93	1.03	1.11	1.18	1.23	1.28	1.33	1.37	1.41	1.44	1.48			
15	2.04	2.27	2.44	2.59	2.71	2.82	2.92	3.01	3.09	3.17	3.24	3.31		
20	3.57	3.96	4.26	4.52	4.73	4.92	5.10	5.25	5.40	5.53	5.66	5.77		
25	5.50	6.10	6.57	6.96	7.29	7.59	7.85	8.09	8.31	8.52	8.71	8.89	9.06	9.23
30		8.69	9.35	9.90	10.38	10.80	11.17	11.52	11.83	12.13	12.40	12.66	12.90	13.13
35			12.60	13.35	13.99	14.55	15.06	15.52	15.95	16.34	16.71	17.06	17.39	17.70
40			16.32	17.29	18.11	18.85	19.50	20.10	20.65	21.17	21.64	22.09	22.52	22.92
45				21.71	22.75	23.67	24.50	25.25	25.94	26.59	27.19	27.75	28.28	28.79
50				26.62	27.90	29.03	30.04	30.96	31.81	32.60	33.34	34.03	34.68	35.30
55				32.02	33.55	34.91	36.13	37.24	38.26	39.21	40.09	40.92	41.71	42.46
60					39.71	41.31	42.75	44.07	45.28	46.40	47.45	48.43	49.36	50.24
65						48.24	49.92	51.45	52.86	54.17	55.40	56.55	57.63	58.66
70							57.62	59.39	61.02	62.53	63.94	65.27	66.52	67.71
75								67.87	69.73	71.46	73.08	74.59	76.03	77.39
80									79.01	80.97	82.80	84.52	86.14	87.68
85												95.04	96.87	98.60
90												106.16	108.20	110.14
95												117.87	120.14	122.29
100													132.68	135.05
105														148.42

MD (cm)	Height (cm)										
	80	85	90	95	100	105	110	115	120	125	130
30	13.35										
35	18.00	18.28	18.55	18.81	19.06						
40	23.30	23.67	24.02	24.36	24.68	24.99					
45	29.27	29.73	30.17	30.59	31.00	31.39	31.77				
50	35.89	36.46	37.00	37.51	38.01	38.49	38.95	39.40	39.83		
55	43.17	43.84	44.49	45.11	45.71	46.29	46.85	47.38	47.90	48.41	48.90
60	51.08	51.89	52.65	53.39	54.10	54.78	55.44	56.07	56.69	57.29	57.87
65	59.65	60.58	61.48	62.34	63.16	63.96	64.73	65.47	66.19	66.89	67.57
70	68.84	69.93	70.96	71.95	72.91	73.82	74.71	75.57	76.40	77.21	77.99
75	78.68	79.92	81.10	82.23	83.32	84.37	85.39	86.37	87.32	88.24	89.13
80	89.15	90.55	91.89	93.17	94.41	95.60	96.75	97.86	98.93	99.98	100.99
85	100.25	101.82	103.33	104.77	106.16	107.50	108.79	110.04	111.25	112.42	113.56
90	111.98	113.73	115.42	117.03	118.58	120.08	121.52	122.91	124.27	125.57	126.85
95	124.33	126.28	128.15	129.94	131.66	133.32	134.93	136.47	137.97	139.43	140.84
100	137.31	139.46	141.52	143.50	145.41	147.24	149.01	150.72	152.38	153.98	155.54
105	150.91	153.27	155.54	157.71	159.81	161.82	163.77	165.65	167.47	169.23	170.94
110			170.19	172.57	174.86	177.07	179.19	181.25	183.24	185.18	187.05
115						192.98	195.29	197.54	199.71	201.81	203.86
120							212.06	214.50	216.86	219.14	221.36

Conclusions

Total height and mean crown diameter are the allometric variables most involved in the estimation of the dry leaf biomass of *Lippia graveolens* in *General Cepeda*, *Parras de la Fuente* and *Ramos Arizpe* municipalities, *Coahuila*.

The Schumacher-Hall model is the one that best predicts the dry leaf biomass (*DLB*) of the standing oregano shrubs and can be used to calculate the usable biomass in the oregano plots of southeastern *Coahuila*.

Acknowledgments

Conflict of interests

The authors declare they have no conflict of interests

Contribution by author

E. Edith Villavicencio-Gutierrez: field data collection, data analysis, drafting and editing of the document; Adrián Hernández-Ramos: data analysis, drafting and revision of the document; Cristóbal N. Aguilar-González: technical input and revision of the document; Xavier García-Caves: drafting and revision of the document.