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Adaptation, Biometric Traits and Performances of Guayule Lines Grown in Two Mediterranean Environments

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Abstract: The perennial shrub guayule (*Parthenium argentatum* A. Gray) has gained interest as a potential source of natural and hypoallergenic rubber in Southern Europe. Although, native to northern Mexico, it is suited to semi-arid and Mediterranean environments. A research study was conducted in Sardinia (Italy) to evaluate adaptation and biometric traits of introduced guayule lines and to determine the contents and yields of rubber and resin obtainable from its aboveground biomass. Seedlings of the accessions AZ-1, AZ-2, P803, and 11591 were field transplanted in 2015 at two locations of southern, and northern Sardinia, respectively, differing for annual precipitation. Plant survival rate, height and width, trunk diameter, leaf chlorophyll concentration and photosystem photochemical efficiency were monitored. Shoots were harvested at 30 months after transplanting and were partitioned into twigs and remaining stems and its rubber and resin contents were determined. Location markedly affected plant survival rates and biometric traits. Dry matter yield of aboveground components as well as contents of rubber and resin and plant rubber and resin yields differed significantly among accessions under comparison. We found that AZ1 and 11591 were the most successful accessions at southern Sardinia site, whereas 11591 was the only accession exhibiting a satisfactory plant survival rate in the northern location.

Keywords: *Parthenium argentatum*; Sardinia; adaptation; measurements; growth; biomass; natural rubber; resin; yield

1. Introduction

Guayule (*Parthenium argentatum* A. Gray) is a woody perennial shrub (50–90 cm high), from the Asteraceae family, indigenous to semi-arid regions of northern Mexico and southern Texas [1–3]. Since the beginning of the twentieth century, this plant has received recurrent attention as one of the most important alternative sources to produce high molecular-weight and hypoallergenic natural rubber [4–6]. The global production of natural rubber is obtained from Hevea [*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.)] trees, which are grown in tropical regions, primarily Southeast Asia countries [2,3]. Particularly in the industrial and medical sectors, natural rubber is of essential and strategic importance in thousand applications, from the production of medical devices to automotive and aircraft tires. Indeed, its unique biopolymer (1,4 cis-polyisoprene) possesses high performance properties, such as resilience, elasticity, abrasion resistance, efficient heat dispersion, impact resistance, and malleability at cold temperatures, which cannot be achieved by synthetic rubber forms [7]. Hevea rubber could cause allergic reactions, whereas guayule is regarded as an under-used source of hypoallergenic latex. Additionally, potential rubber supply shortages from Southeast Asia might be determined by fungal diseases on Hevea and land use changes in areas of production. For the above reasons, the dependence upon Hevea as a single source of natural rubber is risky for all countries that are not producers. Moreover, natural rubber has been listed as a critical material by European Union [8], which is completely dependent upon imported natural rubber sources, amounting to about 1.2 million tons annually [9].

Guayule shrub grows within a temperature range of −15°C to +40 °C, with annual rainfall requirements from 350 to 800 mm. Therefore, it is well suited to semi-arid and Mediterranean regions such as South of France, Italy, Spain, Morocco, and Turkey [10,11]. Currently, it is considered as a potential alternative source to produce natural rubber in Europe with the potential to become commercial crop [12]. According to Snoeck et al. [13], guayule in Mediterranean countries might be commercially profitable if in addition to rubber, the resin and biomass have economic markets (including bioenergy to drive the guayule processing plant) [14]. As stated by the same authors, an overall interest in developing new agricultural commodities, such as guayule for Mediterranean or semi-arid climates is justified. In 2018, the US tire manufacturer Bridgestone Americas and Italy-headed polymer and elastomer producer Versalis S.P.A. established a strategic partnership, in order to develop and deploy a "comprehensive technology package" to commercialize guayule in the agricultural, sustainable-rubber and renewable-chemical sectors (info@bioenergyinternational) testifying the strong interest in guayule.

It is noteworthy that first attempts for produce natural rubber from the guayule plant in Italy dated back to 1933. Field experiments were conducted in Apulia region and Sardinia island, but the outbreak of World War II did not support an industrial exploitation, in spite of the collaboration established between the Italian company Saiga and the American Intercontinental Rubber Company [15]. Sardinia territory was already chosen because its suitability for guayule cultivation. However, Sardinia has microclimates and soil types that can be considered representative for other areas of Mediterranean basin to study the impact of environmental factors on the cultivation of guayule. We hypothesized the environmental diversity might influence guayule performances. A research was conducted in two Sardinian locations to evaluate the adaptation and productive potential of guayule grown under Mediterranean conditions. The specific aims of the research were to; (i) evaluate adaptation and biometric traits of introduced guayule lines; and (ii) determine the growth, biomass production, contents and yields of rubber and resin obtainable from guayule biomass.

2. Materials and Methods

2.1. Locations, Experimental Design and Crop Management

The field experiments were conducted from 2015 to 2018 in two locations of Sardinia (Italy) in a Mediterranean climate with mild winter and rainfall mainly received in autumn and winter. The first site was in southern Sardinia (39°31′ N, 8°51′ E) at the research unit of the Council for Agricultural Research and Economics, having a long-term average annual rainfall of 446 mm and a mean annual air temperature of 17.6 °C. The soil, classified as Typic Fluvaquents, is sandy-clay-loam, with pH 7.8, average nitrogen content of 1.1‰ and phosphorous 16.2 ppm [16]. The second site was in a private farm of northern Sardinia (40°35′ N, 8°33′ E) with an average annual rainfall of 750 mm and a mean annual air temperature of 16.1 °C. The soil, classified as Lithic Xerorthents, is sandy-clay-loam, with pH 8.1, average nitrogen content of 1.6‰ and phosphorous 14.4 ppm [16].

Four guayule accessions kindly provided by the Agricultural Research Service of the United States Department of Agriculture (ARS-USDA) were evaluated in this research, the improved guayule lines AZ-1, AZ-2, the wild material PARL 803 10i (thereafter referred to as P803), and the line 11591, which was developed during the Emergency Rubber Project and used as control [17,18].

Seeds were treated to break dormancy according to Naqvi and Hanson [19] and were sown in wet filter paper. Two-week old seedlings were moved into jiffy pots and afterwards put in plastic bags. Initial seedling development took place in a glasshouse, then, two-month-old seedlings were field transplanted in spring 2015. The experimental design was a randomized complete block with three replicates. Seedlings were transplanted in rows spaced at 1 m and 0.5 m within the row. Each plot consisted of eight rows and additional outer rows as border. Seedlings were irrigated daily during the first week after transplanting and at ten-day intervals in summer months from June to early October by adopting a trickle system. Cumulated total rainfall + supplementary irrigation for the entire 30-month period reached about 1400 and 1800 mm in southern and norther Sardinia site, respectively (Figures 1 and 2). Fertiliser was applied only at the time of field preparation for transplanting when 30 kg N ha^{-1} (as urea) was incorporated into the soil.

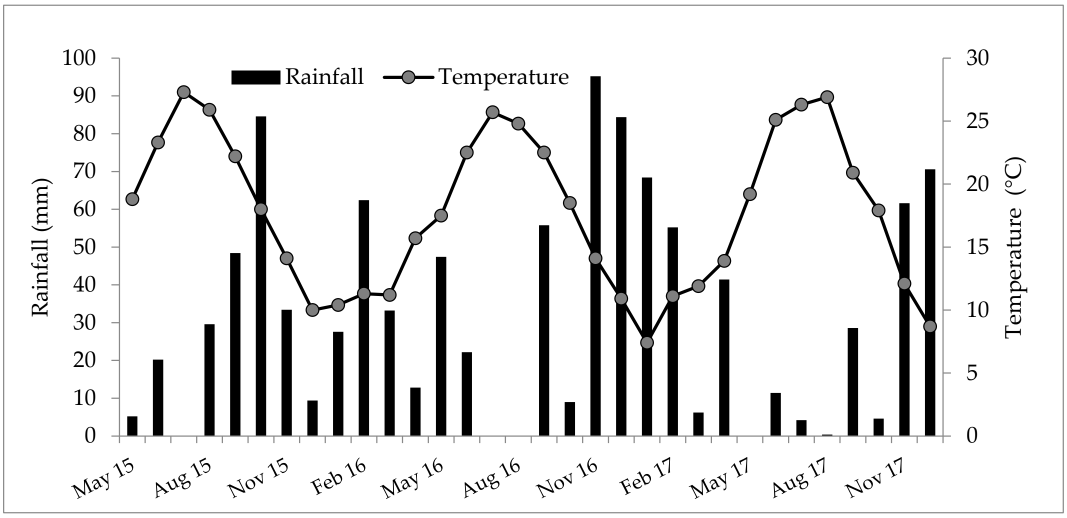

Figure 1. Monthly rainfall and mean temperature at the southern Sardinia site during the study period.

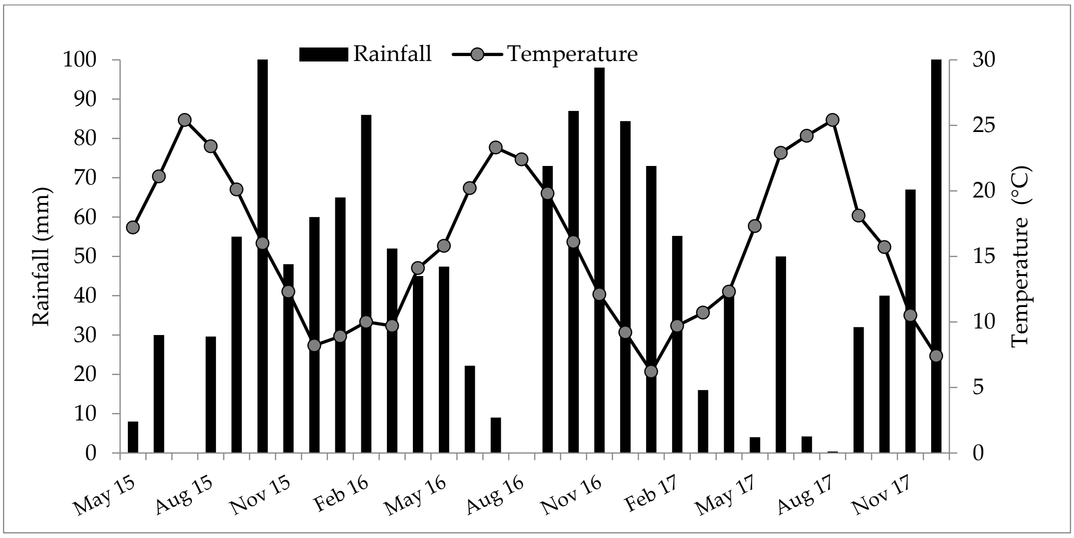

Figure 2. Monthly rainfall and mean temperature at the northern Sardinia site during the study period.

2.2. Measurements of Plant Biometric Parameters, Dry Matter Yield and Sampling

The number of established plants that survived in each plot was recorded at bimonthly intervals. Plant growth and development was monitored by recording plant heights and widths at seasonal intervals. In three representative plants of each line under comparison per plot, plant height was measured by taking the vertical distance from the ground level to the shoot of the tallest branch. Plant width was recorded by measuring the shoot-to-shoot distance of the widest part of the plant (measurements were taken horizontally to the ground surface and perpendicularly to the row). Basal main stem (i.e., trunk) diameter was measured by using a caliper.

On the same three plants per plot, the chlorophyll content on three leaves per plant was estimated with a SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc., Aurora, IL, USA) [20]. Photosystem photochemical efficiency (Fv/Fm) was measured in three fully expanded and exposed leaves per plant, chosen in the upper part of plant using a portable chlorophyll fluorimeter (Pocket Pea Hansatech Instruments, Norfolk, UK) equipped with black leaf clips. Measurements were made between 10.00 AM and 13.00 PM, at 3500 Lmol of PAR m^{-2} s^{-1}. Fluorescence measurements were carried out on the adaxial leaf lamina light after 15 min of dark adaptation period [21,22].

Finally, in winter 2017, corresponding to 30 months after transplanting, three plants of each line per plot were cut at ground level and harvested. Aboveground plant dry matter was partitioned into leaves, twigs (i.e., apical, thin and young stems according to their length, corresponding to last seasonal growth and less than one year old) remaining branches and trunk (thereafter also referred to as stems), which were chipped using a garden shredder to facilitate drying and sampling. Biomass subsamples were oven dried at 60 °C to determine the dry matter content and yield of each plant component. A representative sample of each biomass component (about 100 g per plant) was then ground enough to pass through a 1 mm mesh using a grinding mill and stored before analysis.

2.3. Determination of Rubber and Resin Contents and Yields

The resin and the rubber were sequentially extracted from the tissues of both twigs and stems with organic solvent using an ASE 350 apparatus (Dionex Thermo Fisher Scientific Inc., Sunnyvale, CA, USA). Ground samples of about 1.5 g each were mixed with an equal amount of celite and loaded into 22 mL stainless steel extraction cells containing a Whatman 40 cellulose filter on the bottom. The sample was cover with a cellulose filter and pressed with the filter insertion tool. The cell was filled with glass beads to reduce the internal void space and hand-tighten with the end cap. The extraction conditions for the resin were set as following: temperature 40 °C; two extraction cycles of 5 min each, rinse 100%, and acetone as the solvent. After removal of the resin fraction, the rubber was extracted setting the instrumental parameters as following: temperature 140 °C, two cycles of 20 min each, rinse 100%, hexane as the solvent. The solvent containing the analyte was evaporated under low pressure with a rotary evaporator and the rubber and resin were gravimetrically quantified [23]. Finally, rubber and resin yields were calculated using the product of twig and stem dry weight and its rubber and resin contents.

2.4. Statistical Analysis

For each measurement, data were subjected to a one-way analysis of variance (ANOVA) to test the effect guayule accession on plant height, width, trunk diameter, SPAD, Fv/Fm, dry matter yields, rubber and resin contents and yields. Means were compared based on Tukey's HSD (honestly significant difference) test at the 0.05 probability level. Analysis of variance for dry matter, rubber and resin content and yields was also performed across sites.

3. Results

3.1. Plant Survival, Height and Width, and Trunk Diameter

At 30 months after transplanting, a 100% survival rate of transplanted plants was recorded for the accessions P803 and 11591 at southern Sardinia, whereas mortality in AZ-1 was twice as high as in AZ-2, leading to a final survival rate of about 80 and 90%, respectively (Table 1). At 24 months after transplanting, no AZ-2 plants survived and mortality rate reached 96% for AZ-1 at the northern Sardinia site. Additionally, the final survival rate decreased to 25% in P803 but it was about 60% in 11591.

Table 1. Plant mortality and final survival rate in guayule lines at 30 months from transplanting at the two sites.

Site	Months after Transplanting					
	6	**12**	**18**	**24**	**30**	**Final**
Southern Sardinia			Mortality (%)			Survival rate (%)
AZ-1	0	11	0	11	0	78
AZ-2	0	0	0	11	0	89
P803	0	0	0	0	0	100
11591	0	0	0	0	0	100
Northern Sardinia						
AZ-1	56	20	0	20	4	0
AZ-2	33	0	0	67	0	0
P803	33	17	25	0	0	25
11591	0	11	13	14	0	62

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively.

At southern Sardinia, measurements evidenced slow but quite constant increments in the height of the guayule plants, ranging from a minimum of 33.1 cm (P803) at the first measurement (6 months after transplanting) to a maximum of 58.2 cm (AZ-1) at 30 months after transplanting (Table 2). Plant height significantly differed among guayule lines at 24 and 30 months after transplanting when P803 was lower than 11591, and AZ-1, respectively. On the contrary, only in two accessions out of four, measurements at northern Sardinia evidenced slow but quite constant increments in the height of the guayule plants, ranging from a minimum of 20 cm (P803) at the first measurement to a maximum of 70 cm (11591) at 30 months after transplanting. An opposite trend was recorded for AZ-1 and AZ-2, without substantial increases in plant height after transplanting.

Table 2. Plant height (cm) of guayule lines from transplanting to harvest at 30 months at the two sites.

Site	Months after Transplanting				
	6	**12**	**18**	**24**	**30**
Southern Sardinia					
AZ-1	35.3	53.6	44.7	57.8 a	58.2 a
AZ-2	34.8	53.3	50.3	50.9 ab	52.0 ab
P803	33.1	47.7	51.0	40.3 b	40.4 b
11591	35.1	53.6	54.0	56.4 a	52.9 a
Northern Sardinia					
AZ-1	49.1 a	34.0	47.0 a	35.0	n.a.
AZ-2	31.3 b	22.0	29.0 b	n.a.	n.a.
P803	20.1 b	20.0	28.7 b	42.3	57.3 b
11591	31.3 b	25.1	37.6 b	50.8	70.7 a

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively. Means within a column followed by the same letter are not significantly different at the 0.05 level; n.a. = not available.

As general average, temporal variations in the width of guayule plants indicated twice as high values at 24 months from transplanting compared to initial values at six months, except for P803 (Table 3). In the last sampling, AZ-1 plants reached 73.1 cm, it being significantly larger than AZ-2 and P803 plants. At northern Sardinia, variations in the width of guayule plants indicated 4 to 6-fold high values at 30 months from transplanting than 6 months values ones only for P803 and 11591 and no increases for AZ-1 and AZ-2.

Table 3. Plant width (cm) of guayule lines from transplanting to harvest at 30 months at the two sites.

Site	Months after Transplanting				
	6	12	18	24	30
Southern Sardinia					
AZ-1	33.7	58.5	42.3	67.8	73.1 a
AZ-2	28.2	43.3	31.9	50.0	51.3 b
P803	31.2	58.7	35.9	46.6	46.4 b
11591	27.4	54.9	41.2	56.7	63.8 ab
Northern Sardinia					
AZ-1	32.1 a	22.8 a	37.5	23.0	n.a.
AZ-2	15.5 b	13.5 ab	20.0	n.a.	n.a.
P803	10.4 b	8.8 b	23.4	38.3	65.7
11591	18.2 b	15.8 b	32.9	48.0	77.3

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively. Means within a column followed by the same letter are not significantly different at the 0.05 level; n.a. = not available.

A similar trend was recorded for trunk diameter, which on average varied from 14 to 37 mm at 6 and 24 months after transplanting, respectively, whereas at the last sampling the trunk diameter of 11591 was bigger than that of AZ-2 and P803 (Table 4). In an analogous way, trunk diameter increased two or three times from the first to the last sampling but only in P803 and 11591 accessions at northern Sardinia.

Table 4. Plant trunk diameter (mm) of guayule lines from transplanting to harvest at 30 months at the two sites.

Site	Months after Transplanting				
	6	12	18	24	30
Southern Sardinia					
AZ-1	17.3	22.7	25.2	37.0	33.2 ab
AZ-2	13.9	19.7	21.7	25.6	25.7 b
P803	18.9	22.7	25.1	31.2	26.0 bc
11591	17.9	24.9	27.2	32.8	35.4 a
Northern Sardinia					
AZ-1	14.8 a	15.5 a	25.0 a	20.0	n.a.
AZ-2	12.8 a	11.5 b	14.0 b	n.a.	n.a.
P803	6.7 b	6.9 c	13.1 b	20.3	20.8
11591	11.9 a	13.1 ab	17.4 ab	23.6	22.9

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively. Means within a column followed by the same letter are not significantly different at the 0.05 level; n.a. = not available.

3.2. Leaf Chlorophyll Content (SPAD) and Fluorescence Measurements

Leaf chlorophyll content varied from 52.1 to 65.2 SPAD unit and it was unaffected by the guayule lines except at 24 and 30 months after transplanting, when P803 values were higher than those of AZ-1 and AZ-2 (Table 5). Higher SPAD values might indicate a better suitability of P803 and 11591 to environmental conditions at this site compared to AZ1 and AZ2 as also pointed out by differences in survival rate. At northern Sardinia, leaf chlorophyll content varied from 34.5 to 62.9 SPAD unit and it was affected by the guayule lines at 18 months after transplanting, when P803 and 11596 values were higher than those of AZ-1 and AZ-2, indicating a better suitability of P803 and 11591 to the site conditions.

Table 5. Leaf chlorophyll content (SPAD units) of guayule lines from transplanting to harvest at 30 months at the two sites.

Site	Months after Transplanting				
	6	**12**	**18**	**24**	**30**
Southern Sardinia					
AZ-1	53.0	53.0	55.7	52.4 c	55.1 b
AZ-2	52.1	52.1	64.4	56.8 bc	55.1 b
P803	63.3	63.0	59.2	65.2 a	60.8 a
11591	60.8	54.3	62.5	61.3 ab	57.4 ab
Northern Sardinia					
AZ-1	34.5	51.9	37.5 b	31.1	n.a.
AZ-2	48.1	61.0	38.3 b	n.a.	n.a.
P803	41.2	62.9	46.2 a	44.2	56.9
11591	44.4	61.7	48.7 ab	43.3	55.0

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively. Means within a column followed by the same letter are not significantly different at the 0.05 level; n.a. = not available.

Absolute range of leaf chlorophyll fluorescence varied from 0.615 to 0.826 in AZ-1 and AZ-2, respectively. Significant difference among lines were found in the last two samplings, showing lowest values in P803, which were not different from AZ-2. (Table 6). At northern Sardinia, leaf chlorophyll fluorescence varied from 0.335 to 0.840 in P803 and AZ-1, respectively. Significant difference among lines were found in the third sampling, indicating lower values for AZ-2. Unusual and lowest values were reached in P803 at 30 months.

Table 6. Leaf chlorophyll fluorescence (Fv/Fm) of guayule lines from transplanting to harvest at 30 months at the two sites.

Site	Months after Transplanting				
	6	**12**	**18**	**24**	**30**
Southern Sardinia					
AZ-1	0.615	0.806	0.800	0.787 a	0.825 a
AZ-2	0.721	0.826	0.818	0.725 ab	0.812 bc
P803	0.668	0.813	0.820	0.658 b	0.811 c
11591	0.623	0.823	0.803	0.793 a	0.823 ab
Northern Sardinia					
AZ-1	0.783	0.840	0.840 c	0.760	n.a.
AZ-2	0.735	0.830	0.795 c	n.a.	n.a.
P803	0.729	0.806	0.822 b	0.827	0.335 b
11591	0.743	0.824	0.838 ab	0.785	0.563 a

The numbers, 6, 12, 18, 24 and 30 correspond to December 2015, June 2016, December 2016, June 2017, December 2017, respectively. Means within a column followed by the same letter are not significantly different at the 0.05 level; n.a. = not available.

3.3. Aboveground Biomass Fractions, Content and Yield of Rubber and Resin

Statistical analysis evidenced that dry matter yield of aboveground components, contents of rubber and resin and yields of rubber and resin per plant differed significantly among guayule accessions (Table 7). At the site of southern Sardinia, the lowest values of twigs and stems dry matter yield were recorded for AZ-2. Except for P803, twig dry matter represented about one third of the cumulative twigs and stems dry matter yield per plant. On average, rubber contents were in the range 6.9–9.1 % with differences among accessions for stems having different age. Cumulative rubber yields reached 27.3, 8.1, 22.7 and 28.9 g plant^{-1} in AZ-1, AZ-2, P803, and 11591, respectively, and the corresponding potential yields ranged from 160 to 576 kg ha^{-1}. The rubber produced in twigs represented, 35, 30, 65 and 47 % of the total twigs and stems rubber per plant, in AZ-1, AZ-2, P803 and 11591, respectively. On average, resin content was in the range 9.1–10.4 % in twigs and stems, respectively, with differences among accessions. The amount of resin produced in twigs represented 39, 39, 29 and 30 % of the total twigs and stems resin per plant, in AZ-1, AZ-2, P803 and 11591, respectively. Cumulative resin yields were 38.2, 10.8, 29.3 and 30.3 g plant^{-1} in AZ-1, AZ–2, P803 and 11591, respectively. The corresponding potential yields ranged from 215 to 764 kg ha^{-1}.

Table 7. Aboveground biomass fractions, rubber and resin content and yields of plants at 30-month growth at the two sites.

	DM Yield g Plant^{-1}		Rubber Content %		Resin Content %		Rubber Yield g Plant^{-1}		Resin Yield g Plant^{-1}	
	Twigs	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**
Southern Sardinia										
AZ-1	116.7 a	230.5 a	8.2 c	7.7 b	12.6 a	10.2 b	9.6 a	17.7 a	14.7 a	23.5 a
AZ-2	40.8 b	67.0 c	5.8 d	8.5 a	10.2 b	9.8 b	2.4 b	5.7 b	4.2 c	6.6 b
P803	126.5 a	174.5 b	11.7 a	4.5 d	6.7 c	12.0 a	14.8 a	7.9 b	8.4 b	20.9 a
11591	129.9 a	215.8 a	10.5 b	7.1 c	7.0 c	9.8 b	13.6 a	15.3 a	9.1 b	21.2 a
Northern Sardinia										
P803	88.3 b	113.5 b	5.2	5.1 a	4.7 b	4.9 b	4.6 b	5.8	4.1 b	5.6 b
11591	127.0 a	192.5 a	5.4	3.8 b	9.0 a	10.1 a	6.9 a	7.3	11.4 a	19.4 a

Means within a column followed by the same letter are not significantly different at the 0.05 level.

At the site of northern Sardinia, dry matter yields, contents of rubber and resin and yields per plant differed significantly between guayule accessions, which were able to survive until 30-month harvest. Twig dry matter represented about 40 % of the total twigs and stems dry matter yield per plant. On average, rubber content was 5.3 % in twigs and did not reach 4 % in stems of 11591. Cumulative rubber yields varied from 10.4 to 14.2 g plant^{-1} leading to negligible potential yields per hectare. Resin content was in the range 4.7–10.1 % DM in twigs and stems, respectively, it being significantly higher in 11591. The amount of resin produced in twigs represented from 30 to 40 % of the total twigs and stems resin per plant. Cumulative resin yield per plant in 11591 was 3-fold higher than P803.

3.4. Across-Site Comparison

Across-site analysis revealed not significant different dry matter yields per plant as well as higher contents of rubber and resin at the southern Sardinia site in twigs and stems, respectively (Table 8). Cumulative rubber yields varied from 12.4 to 25.5 g plant^{-1}, resulting in about 2-fold rubber yield per plant at southern Sardinia. Cumulative resin yields varied from 18.8 to 30.1 g plant^{-1}.

Table 8. Means of aboveground biomass fractions, rubber and resin contents and yields of P803 and 11591 lines at the two sites.

	DM Yield g Plant^{-1}		Rubber Content %		Resin Content %		Rubber Yield g Plant^{-1}		Resin Yield g Plant^{-1}	
	Twigs	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**	**Twigs**	**Stems**
Southern Sardinia	128.2 a	195.2 a	11.1 a	5.8	6.9 a	10.9 a	14.2 a	11.3 a	8.8 a	21.3 a
Northern Sardinia	107.7a	153.0 a	5.3 b	4.4	6.8 a	7.5 b	5.7 b	6.7 b	7.3 a	11.5 b

Means within a column followed by the same letter are not significantly different at the 0.05 level.

4. Discussion

Although it is native to northern Mexico and southwest regions of the USA, guayule is considered suitable to semi-arid and Mediterranean regions [11]. Currently, there is increasing interest in it, as an alternative source to produce natural rubber in Southern Europe [12]. Notwithstanding the acknowledged potential suitability of this shrub species, very few papers document the adaptation and performances, over time, of guayule accessions, introduced in Mediterranean areas, which are featured by large environmental and biological diversity.

The current study is one of the first reports regarding bioagronomic traits and quantification of rubber and resin contents and yields from both twigs and branches of guayule plants grown in Mediterranean environment. Despite the first attempts carried out in Italy to grow guayule before the outbreak of World War II [15], no information is available in literature on guayule adaptation to Italian environmental conditions, to the best of our knowledge. The only available information for southern European areas comes from the long-term activity on guayule carried out by the French Cirad (Centre de coopération internationale en recherche agronomique pour le développement) at Montpellier (France). Within the EU-Pearls project [24], a specific workpage dealt with guayule germplasm, breeding, and agronomy. Field experiments have been carried out at Montpellier (France) and Cartagena (Spain), which are two sites differing for climatic traits (a mean annual rainfall of 776 and 350 mm, respectively). Therefore, Sardinian sites having 450 to about 750 mm of precipitation can be considered quite comparable with the abovementioned Spanish and French sites. First, our results testify the importance of environmental conditions on the survival of guayule plants, indicating a lower adaptation capacity in the improved lines AZ-1 and AZ-2 compared to 11591 at the site of northern Sardinia. Higher rainfall coupled with a poor soil drainage and/or occasional frost events might presumably favoured root diseases, causing a high plant mortality at that site, reducing possible comparison with other results. Snoeck et al. [25] reported an overall higher adaptation of guayule germplasm (both Mexican and US accessions) in the drier site of Cartagena, whereas frost damage on plants was recorded in Montpellier where irrigation was conversely unnecessary because rainfall was already high. Plant survival, rubber content, dry matter and rubber yields were affected by genotype, site and adopted management in terms of irrigation and fertilization. However, rubber content of stems at Cartagena reached 6.8% as in our results, very different from values of Montpellier site (about 4%).

Outside southern Europe, Dissanayake et al. [26] evaluated improved lines of guayule in a black earth soil at a sub-tropical climate site of Australia. They reported a rubber and resin yield increase in the range of 53–123% for lines AZ-1 and AZ-2 over 11591. In contrast to Dissanayake et al. [26], we recorded quite similar or even higher values for 11591 compared to AZ-1 and AZ-2. The different duration of growth period from transplanting to harvesting and adopted management interventions in the different experiments, make it difficult to compare the same guayule accession grown worldwide. On the other hand, improved lines were developed for specific and targeted USA areas and not for a global spreading of the guayule cultivation [17]. Additionally, proper comparisons for the same accessions carried out at the same or similar plant age (i.e., 24 or 30 months after transplanting) are quite limited.

Dissanayake et al. [27] also evaluated the same accessions in two distinct Australian sites and found higher rubber and resin contents for AZ-1 and AZ-2 in the drier site, but similar yields in both sites. They found very lower rubber and resin yields for 11591 in the drier site, even though rubber and resin contents were higher. Compared to the Australian results, our values were lower for AZ-1 or very lower for AZ-2, but similar or higher for 11591. Potential rubber yields were overall lower than our values for the same 11591 accession when grown in Argentina [28] where the different season at harvest had little effect on yield performances. Not surprisingly, rubber and resin yields were also affected by plant dry matter yields, which also varied in the different experiments. More recently, the performances of several guayule lines were investigated in the Western Cape regions of South Africa [29] indicating a successful establishment of guayule lines AZ-1 and 11591 as in our research. Additionally, field measurements carried out on 2-year old plants allowed direct comparisons with our results for some biometric traits. At the Western Cape experimental farm, AZ-1 and AZ-2 reached a plant height of 90 cm and were taller than in Sardinia, whereas plant height values for 11591 were quite similar. On the contrary, main stem diameters for AZ-1, AZ-2 and 11591 lines in Sardinia were twice as larger as in South Africa. The comparisons for biometric traits with Australian results [27] showed quite similar plant height values for AZ-1 and 11591, whereas higher values for AZ-2 leading to taller plants than those recorded in southern Sardinia. However, in that Sardinian site AZ-2 and 11591 did not significantly differ for plant height.

Regarding leaf chlorophyll contents (measured as SPAD units) at northern Sardinia site, P803 showed the highest values that were significant different from AZ-1 and AZ-2 but not from 11591. Unfortunately, direct comparisons for leaf chlorophyll contents and leaf chlorophyll fluorescence (Fv/Fm) are not possible for the same accessions grown in field. However, P803 accession differed significantly from remaining ones under comparison for Fv/Fm and the AZ-2 value, not different from P803, was lower than AZ-1 at 30-month sampling. Overall, our values recorded at 12, 18 and 30-month sampling were slightly higher than values obtained in hydroponically grown guayule plants or its shoot-regenerated cultures [30,31]. It is worth noting that the leaves of P803 were very small and with a different shape compared to AZ-1, AZ-2 and 11591. In healthy non-stressed plants, Fv/Fm is around 0.83, while any type of stress that results in inactivation or damage to photosystem II (photoinhibition) causes a decreasing of Fv/Fm value [20]. Therefore, our measurements of chlorophyll fluorescence in vivo, and on intact and attached leaves, indicated differences among accessions under comparison, as well as possible associated stress conditions. According to Veatch and Ray [32] difference in photosynthetic rates could account for the increase of rubber production in the winter months.

A recent comparison across six sites in Arizona and Texas evaluated the phenotypic variations in dry biomass, rubber and resin content and production in nine improved guayule germplasm [33]. The reported mean rubber and resin yields for 2-year old plants of 11591 were almost coincident with our values, even if rubber and resin contents differed. On the contrary, mean rubber and resin yields were twice as high as our values for AZ-1 and almost 4-fold than our values for AZ-2. It is worth noting that large variation was found for the same trait. For example, AZ-2 rubber content ranged from 2.05 to 4.80% in individual locations as well as resin yield varied from 791 to 2207 kg ha^{-1}, and so on. Authors pointed out that the investigated guayule germplasm has a good genetic variability in biomass rubber and resin production also suggesting the possibility of selection for more than one trait at a time. Finally, the potential rubber and resin yields from Abdel-Haleem et al. [33] were quite conservative compared to those indicated by Luo et al. [34]. Derived from an experiment carried out in Arizona for estimating the potential breeding values for different guayule accessions (as Best Linear Unbiased Prediction), the potential rubber yield of 1200 kg ha^{-1} for 11591 and about 900 kg ha^{-1} for both AZ-1 and AZ-2, respectively, were reported [34].

Based on the above reported comparisons within global trials, it might be stated that the accession 11591 performed better in regions of Texas, France, South Africa and now Italy. However, it did not occur in Argentina and Australia. The worldwide performances of AZ-2 have always overtaken our results. Such differential yields between countries may be accounted for by genotype x environmental interaction, where the specific combination of different factors, namely soil type, temperature, rainfall, irrigation, is important. In summary, our findings indicate a low suitability of AZ-1 and AZ-2 to be grown at the environmental conditions of the northern Sardinia site, where 11591 had the highest survival rate, and the limited performances of the AZ-2 accession at the conditions of the southern Sardinia site, not in agreement with reports from other countries. Unfortunately, no comparative values are available in literature for the accession P803, to the best of our knowledge. However, based on the divergent information from worldwide reports and taking into account the environmental variability of Mediterranean climatic areas, caution must be used when extrapolating our information for other environments of Mediterranean basin, which are not necessarily similar for soil type and annual rainfall amounts. For the same reasons, additional fields experiments are required country by country in order to ascertain the possibility of guayule cultivation across its potential cultivation area in Mediterranean

Europe and/or Mediterranean basin, which is characterized by a diversified gradient of land suitability for guayule cultivation, as already indicated [12].

Finally, traditional farming systems are important at the same Mediterranean areas and therefore, it is preferable, at least as a valuable option, for the integration of guayule crop into existing farming systems. As evergreen drought resistant shrubs might alleviate critical fodder shortage, the forage potential of guayule leaves is also being investigated [35]. This approach will contribute to exploitation of the whole plant biomass and non-rubber co-products not only for biobased by-products [14,36–38], but also for complementing traditional activities. Additionally, it might contribute to support the interest in developing new agricultural commodities such as guayule for Mediterranean regions over the next years.

5. Conclusions

The results pointed out different survival rates and variations in plant biometric traits, as well productive performances among guayule accessions introduced in two Mediterranean environments of southern Europe. Dry matter yield of aboveground components, contents of rubber and resin and plant rubber and resin yields differed significantly among accessions. Our findings evidenced that AZ-1 and 11591 were the most successful guayule accessions at the southern Sardinia site, whereas 11591 was the only accession having a satisfactory plant survival rate at the northern Sardinia site. Overall, our data suggest the need of testing additional accessions in multisite experiments to cope with environmental variability and to identify the most critical factors for the adaptation and cultivation of guayule in Mediterranean areas.

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