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Progress of guayule trials in Europe (Spain and France): early evaluation

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Abstract

The main objectives of the project were to define the potential for cultivation of guayule (*Parthenium Argentatum*) in Southern Europe and to assess the economical justification as an alternative source for natural rubber produced in Europe.

The best available subsets of guayule lines from Arizona germplasm were collected from USDA, National germplasm and US Universities (Arizona, Texas). The seeds from the germplasm collection both in France and Spain were used to produce enough guayule plants for local field trials and to settle a guayule collection of selected accessions to produce seeds for European projects. The genetic diversity of 40 guayule imported varieties was tested in two locations: Murcia in Spain, and Montpellier in France. The results showed clear genetic differences. First results showed that Mexican varieties are best adapted to South of Spain. Germination rates of seeds produced by the project varied from 5% to 60% depending on origin, age, line and seeds cleaning.

A fertilization and irrigation trial was set-up in Murcia and Montpellier in May 2009. Three levels of irrigation and 3 levels of fertilization were compared using AZ2 seeds as genetic material. In Montpellier, different levels of irrigation did not alter the growth, but less watering reduced the mortality rate. While in Murcia, watering resulted in significantly higher yields.

The low rubber content (3.2% in March 2010) and a high mortality of the plants (> 60%) observed in Montpellier after the 2009-2010 winter showed that France is not yet adapted for commercial cultivation of guayule.

Rubber content of guayule plants in Murcia (Spain) harvested was 7.4% in March 2010 (for 17-months-old plants). Less than 1% mortality was observed, and the average yields of the irrigation trial was above 10 tons of dry matter (i.e.: 700 kg/ha of rubber) after two years, showing that the area is fully adapted for commercial cultivation of guayule, provided good watering.

Introduction

Demand for rubber is increasing with global development. Currently, the main source of natural rubber is from Hevea which is a tropical crop; hence the idea to develop other sources of production adapted to other climates. The EU-Pearls project aims at the development of rubber production in Europe in order to provide a source of rubber complementary to that of Hevea rubber. Guayule (*Parthenium argentatum*) is one of these alternatives.

Guayule is a shrub native to the deserts of northern Mexico and southern USA, which produces high quality rubber. Despite its low water and labour needs and the early development in the U.S. (since the First World War), guayule production remains low.

The objective of this work supported by EU-Pearls was to determine the viability of guayule cultivation in the Mediterranean regions of Southern Europe for a possible establishment of large-scale production. For this purpose, we explored the growth potential of guayule in two contrasted Mediterranean regions from the varieties obtained from the U.S. germplasm and we tried to define the best cultivation practices. The amount of latex and resin in the different tissues of the plants (leaves, branches, roots) on the studied varieties at different stages of their development was determined in the material harvested.

Favourable climate and soil conditions

Studies in the U.S. and Mexico (1, 2, 3, 4, and 5) have helped defining the climate requirements of guayule and main farming practices: irrigation, fertilization, weeds control, and plant reproduction.

Guayule supports high temperatures (> 40 ° C). In contrast, a cold climate seems to be a negative factor in its development and temperatures below 0 °C can cause high mortality, and is responsible of limited and slow production of biomass.

Recent studies have shown that the minimum rainfall is 200 mm, but from 1000 to 1300 mm (well distributed) are required to achieve maximum production.

Guayule root system is specific to desert soils. Thus, these must be well-drained soils with a high proportion of sand or gravel (clayey soils are unfavourable). Despite its desert origins, it requires large amounts of water at planting and regular irrigation during growth to maximize production. However, it does not withstand water-logging. The water salinity can be a negative factor in case of high concentration during the crop establishment and during its growth. Indeed, the salt causes greater soil resistance to water absorption. The acceptable limits appears to be 1 dS/m at planting and 4.5 dS/m during growth.

Latex production is increased by environmental stresses such as water stress. Mastering this factor is therefore necessary to optimize production.

Weeds control is part of the necessary actions when cultivating a slow-growing shrub, especially during the establishment period. Weeds control can be reduced when the guayule has grown and completely covers the plot. Pre-emergence treatments are currently used in the U.S.; they are toxic to guayule and therefore, they should be applied before planting or during the period of winter dormancy for already established plants. In Europe, the use of plastic mulch was preferred for the first trials.

Trials

For the field trials, we selected two sites with contrasting Mediterranean climates, but both are meeting the climatic requirements of guayule: Lavalette station in Agropolis, near Montpellier (France) and El Molinar station, near Cartagena (Spain).

- Montpellier has average temperatures ranging from 5 - 7 °C in winter to 21 - 24 °C in summer. Average total annual rainfall is 776 mm, with 3 dry months (P_{mm} / $T_{°C}$> 2).

- Cartagena has average temperatures ranging from 10 - 12 °C in winter to 22 - 28 °C in summer. Average total annual rainfall is 300 mm, with 7 dry months (P_{mm} / $T_{°C}$> 2).

Three trials were conducted in both sites, using the same statistical designs. They are:

- A germplasm trial with objectives of 1) defining the cultivars best adapted to the Southern European climates; and 2) producing locally the seeds of selected guayule cultivars for further European trials or development projects.

- A fertilization and irrigation trial to test these two major farming practices.

- A variety trial to statistically assess the behaviour of the best varieties arising from the germplasm. This trial was only started in 2010 and will not be reported here.

Germplasm trial

Material and methods

The seeds were provided by the University of Arizona from the germplasm distributed by the US National Germplasm System (NGPS) for educational and agricultural research, by Yulex, and by USDA. The varieties are of Mexican origin (Coahuila, Zacatecas) and U.S. origin (California, Arizona, and Texas). During the first year of the EU-Pearl project (2008), a total of 40 cultivars were planted in France and 24 in Spain.

Statistical design: The trial was laid out as a randomized block design. The field was divided in elementary plots with 4 cultivars × 50 plants (200 plants / plot). Each plot was limited with two border-lines and 2 border-plants (i.e. 92 plants for observations).

Observations: We observed germination rates, height, weight of each part of the plants, and plant mortality after winter (for Montpellier). The rubber and resin contents were measured at two stages (March and October) using AES extraction conditions: Acetone 40°C (3 cycles of 20 minutes each), Hexane120°C, (3 cycles of 20 minutes each).

Nursery and planting: Guayule seedlings were grown in nursery for 2 months in polystyrene pots filled with peat and vermiculite. Planting was done on 80-cm beds covered with plastic mulch and drip irrigated. Line spacing was 0.5 m and within-row plant distance was 0.4 m. The seedlings were planted in the field in August 2008.

Field results in Montpellier

Dry matter

Five plants of each cultivar were harvested at 17 months, after the winter cold period (March 2010). The leaves were removed and the roots and shoots (stems and twigs) were weighted separately. Fig. 1 shows the average dry matter yields for each studied cultivar expressed in tons/ha assuming a planting density of 50,000 plants/ha (0.4 m × 0.5 m).

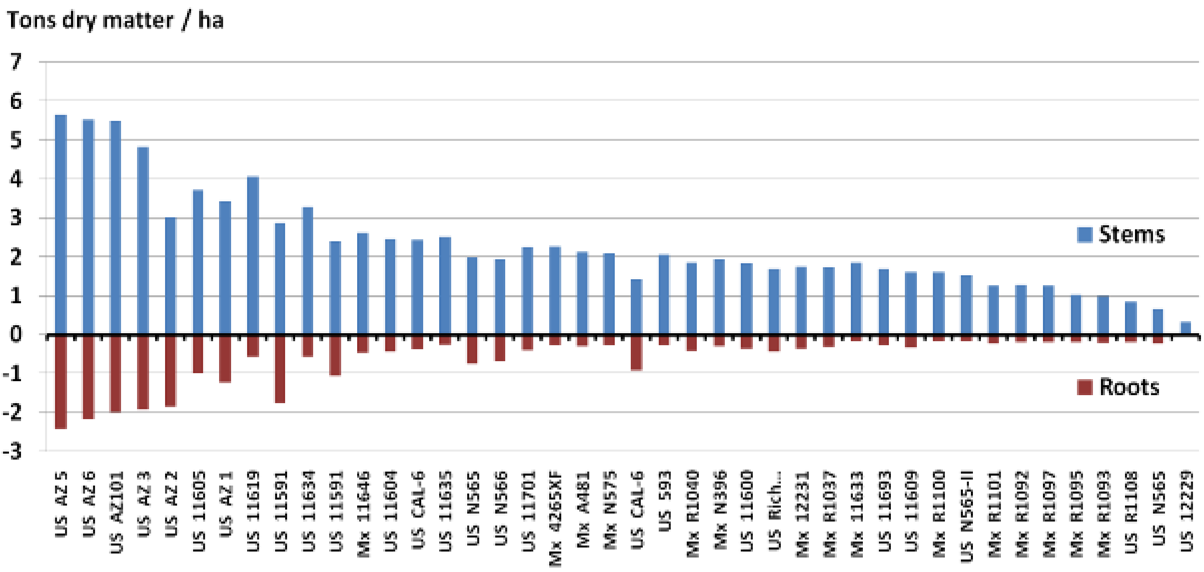

Fig. 1 Roots and shoots dry matter of various guayule origins. US is for U.S. origins and Mx for Mexican origins. Plant density was 50.000 plants/ha.

AZ lines gave good yields, with the best yield obtained for AZ 5 which produced 8.1 tons/ha (roots plus shoots). Three of the cultivars produced less than 1 ton/ha.

Plant height followed the same pattern as biomass. Fig. 2 shows the high correlation between these two variables.

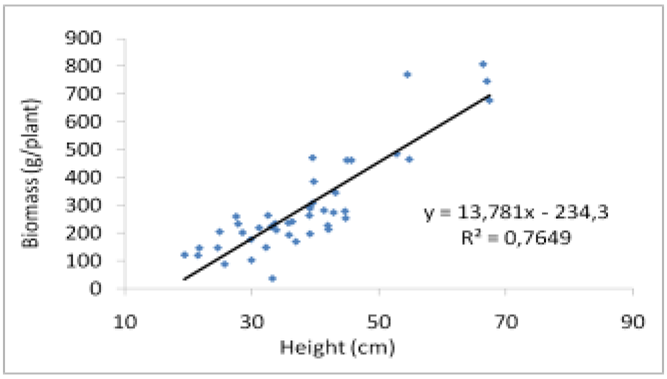

Fig.2 Relationship between biomass and plant height in April 2009

Rubber and resin contents and rubber yields

The percentage of rubber contents was measured in the shoots of 16 selected cultivars. The results are shown in Fig. 3 (rubber) and Fig. 4 (resin).

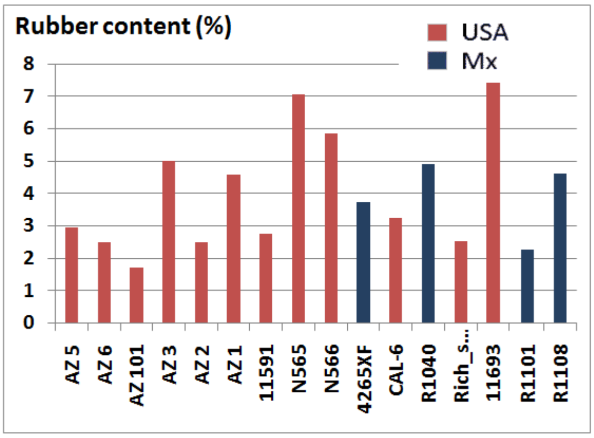

Fig. 3 Rubber content in shoots of 16 selected varieties at 17 months (March 2010)

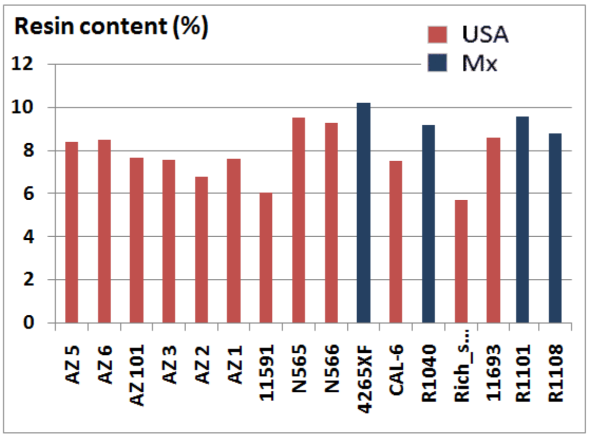

Fig. 4 Resin content in shoots of 16 selected varieties at 17 months (March 2010)

The average rubber content in shoots was 3.98 % with significant differences between the cultivars.

The average resin content was 8.22 % with significant differences between the cultivars.

Resin and rubber contents were poorly correlated. This was particularly true for R1101, 4265XF, AZ 5, AZ 6, AZ 101.

The rubber content in the cultivars (Fig. 3) was not correlated with the dry matter yields (Fig. 2). Thus, the ranking of cultivars based on their rubber yields was different from that of the best plant growth. Fig. 5 shows the rubber yields obtained after multiplying the percentage of latex by the dry matter for the 16 selected cultivars.

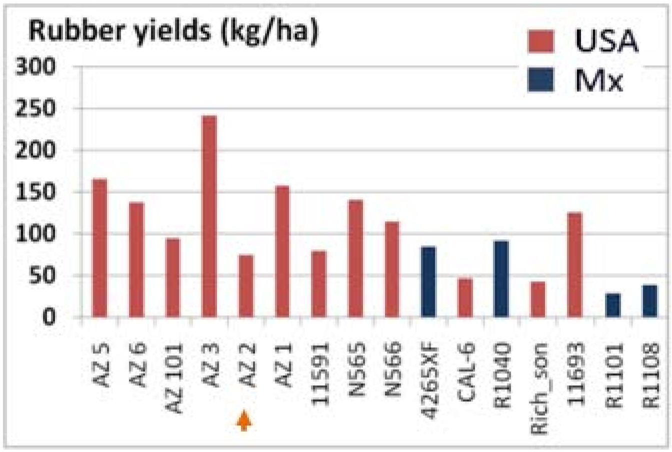

Fig. 5 Rubber yields in shoots of 16 selected varieties after winter at 17 months (March 2010)

AZ 5, AZ 6, and AZ 101 had best dry matter, but low % of rubber content, thus maximum rubber yields attained was not higher than 250 kg rubber/ha.

Effect of adverse climate and soil conditions

The above results were obtained from the computation of yields taken from five living plants taken randomly in the field plots. They do not take into account the actual number of plants alive in the field. In the case of the Montpellier site, the poor climatic conditions of the 2009-2010 winter and spring had adverse effects on the plants survival, and this factor cannot be neglected. Fig. 6 shows the percentage of surviving plants per cultivar after the 2009 winter (average climatic year) and after the 2009-2010 winter which was particularly cold and wet. Indeed, last winter and spring 2010 were particularly cold (53 days below 0 °C) and rainy with a total of 357 mm for the winter period instead of 151 mm on average year.

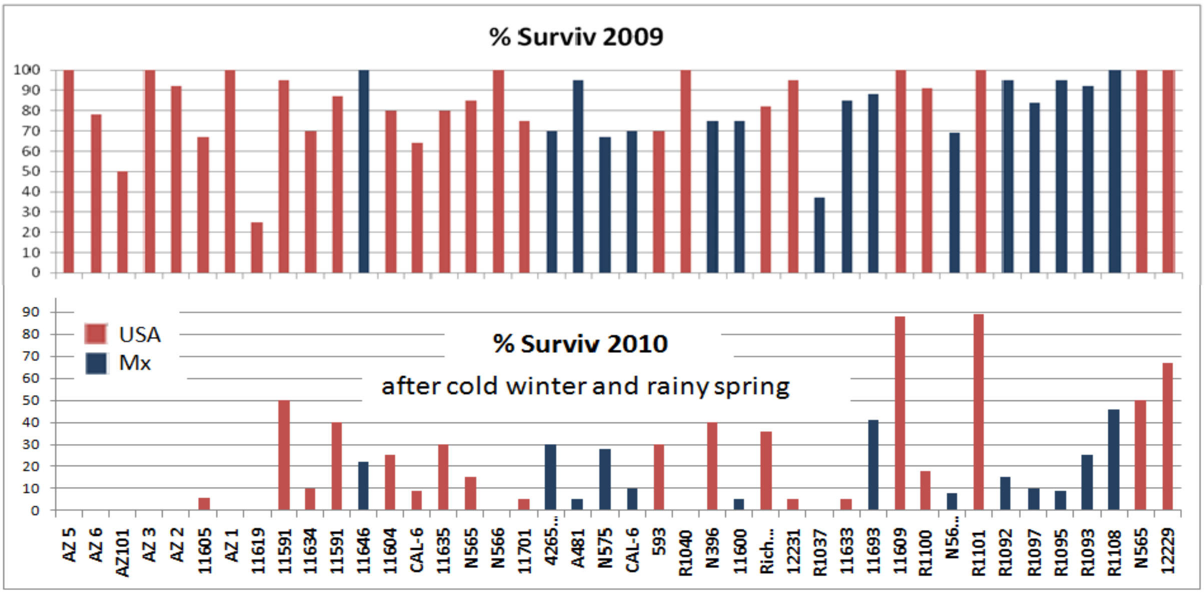

Fig. 6 Survival rates after 1 year and after 2 years

The average survival rates were good in 2009, with 100 % of the cultivars still present after winter, but with varying percentage of surviving plants within a line (from 100% to 25 %).

After the 2010 cold winter and rainy spring, we still had 76 % of the cultivars still present, but with only 88% to 5 % of surviving plants within in a line. Most of the missing lines are of US origins.

The 11591 Line was repeated twice and both treatments showed good performance against the cold weather.

Field results in Murcia

Five plants of each cultivar were harvested after 17 months, in March 2010. The roots, shoots (stems and twigs) and leaves were weighted separately in fresh and dry matter.

The average percent of dry matter per fresh weight was 55.2% with genetic variation not statistically different between cultivars.

Fig. 7 shows the average dry matter yields of each cultivar at 17 months expressed in tons/ha assuming a planting density of 50,000 plants/ha.

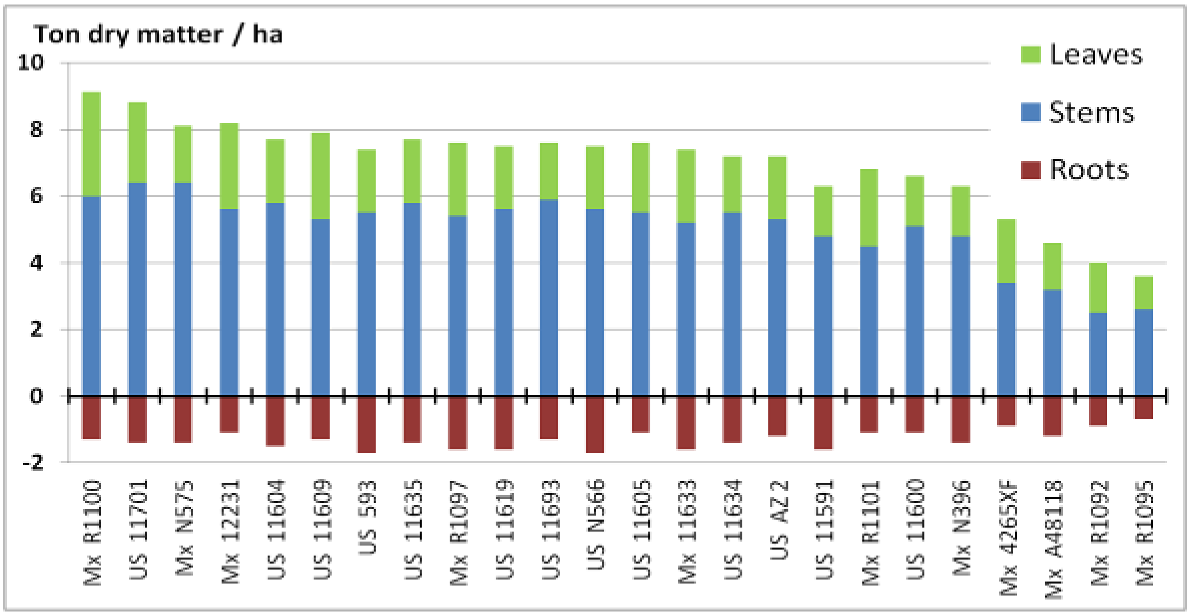

Fig. 7 Roots, shoots, and leaves dry matter of various guayule origins. US is for U.S. origins and Mx for Mexican origins. Plant density was 50.000 plants/ha

The dry matter yields showed significant variations on 17-months-old plants. The ranking shows that 4 Mexican varieties were in the top 5. Line R1100 gave the highest total dry yield, with 10.9 t/ha; whilst line R1095 gave the lowest yields which were 4.2 t/ha only. Differences in shoot, leaf, stem and root yields basically followed the pattern of total dry matter yield, as all dry weight yields were positively correlated (and some very highly). As shoot dry weight made up about 85 % of the total dry weight, the variation in total dry weight was, for more than 98 %, determined by the variation in shoot dry weight. Genetic differences in dry matter distribution are statistically significant, but not very large.

The average percent rubber content was 7.38 % with some plants yielding up to 9 % rubber. These results are high compared to Montpellier (less than 4 %).

Plant height followed the same pattern as biomass. Fig. 8 shows the high correlation between these two variables.

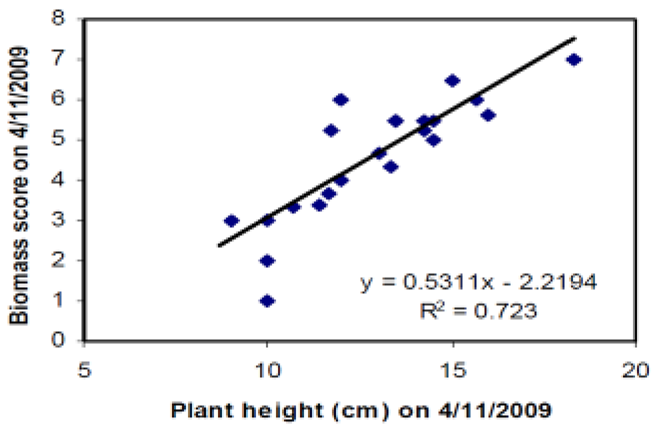

Fig. 8 Relationship between biomass and plant height in April 2009

The climate and soil of the area were adequate for guayule cultivation and less than 1 % mortality was recorded during the two first years of the project.

With these results, yields of more than 700 kg rubber / ha can be expected from 2-years-old plants.

Setup of a user guide for preparing seeds

One of the objectives of the project was to enable us to produce our own seeds from the germplasm planted in Europe.

Guayule reproduces predominately by apomixis (asexual reproduction by seed). The seed is an achene attached to a bract and a pair of sterile flowers, which makes it difficult to process, because most seeds are not filled or not viable and only 10 to 45% of them give a plant.

A protocol was setup using the seeds harvested during the growth period of May to October 2009.

To prepare seeds for germination and planting, we established the following procedure:
1. Collect seeds and sort them roughly;
2. Extract the seeds from their husk by rubbing between 2 hard and corrugated rubber plates;
3. Sieve through various size of mesh (from 1.4 to 0.6 mm);
4. Collect the "> 0.8 mm" fraction by aspiration with a vacuum cleaner;
5. Keep heavy seeds using densimetric table.

With this method, 100 g of raw seeds collected and sorted are needed to produce 6 g of dense filled seeds.

A test of germination was performed with the seeds obtained to validate this method for some selected cultivars (Table 1).

Table 1: Average germination rates of some varieties prepared in France

Variety	Sowing date	Germination rate after 6 weeks (%)
AZ 2	15/01	39
CAL 6	15/01	57
11591	15/01	27
N 565	15/01	46
593	15/01	60

From 27 to 60% of germination rate could be obtained with this method, which is good compared to the average rates (10 to 70%) found in the literature (Jorge M.H.A).

With the protocol developed, we could use the seeds harvested in 2009 to plant further trials: ferti-irrigation trial in 2009 and cultivars trial in 2010.

Fertilisation & irrigation trial

Material & Methods

The trial was planted in April 2009 with AZ 2 nursery grown seedlings.

The statistical design was the same for both sites (Montpellier and Murcia):

- Irrigation: 3 levels (33%, 66%, 100%) *at 100% fertilization.*
- Fertilization: 3 levels (0%, 50%, 100%) *at 100% irrigation.*

Planting was done on 80-cm beds covered with plastic mulch and drip irrigated. Line spacing was 0.5 m and within-row plant distance was 0.4 m. The seedlings were planted in the field in April 2009. Piezometers were used to control soil humidity.

The 100 % irrigation (15 mm if soil moisture > 30 cbars) and 100% fertilization (50 kg N/ha/yr in 2 applications) treatment was common and used as control. The treatments were repeated randomly in three blocks.

Observations were done on the height and weight (not for the first year) of plants, the number of plants after summer and winter (Montpellier). The rubber and resins contents were measured at two stages: 6 (after summer) and 12 months (after winter).

Field results in Montpellier

The height of plants was the main parameter used to measure the growth of plants for the first year. Table 2 shows the average plant heights measured after 6 and 12 months.

Table 2: Plant heights were not statistically different between treatments

Irrigation	Fertilisation	Height 2009	Height 2010
33	100	30.3	31.8
66	100	31.7	35.0
100	*100*	*30.7*	*30.0*
100	50	32.3	34.9
100	0	31.3	36.6

The plant heights were not statistically different between treatments. The applications of irrigation and fertilization did not affect the growth parameter.

The percentages of rubber were not statistically different between treatments. The following averages were obtained: Leaves = 1.51%; Stems = 2.61%. The percents of resin were not statistically different between treatments. Average was 7.00 % resin in shoots.

As for the germplasm trial, the climate of Montpellier had a negative effect on plant survival. The number of surviving plants after summer and winter was recorded (Fig. 9).

The treatments with less irrigation gave significantly better survival rates after the cold winter and rainy spring.

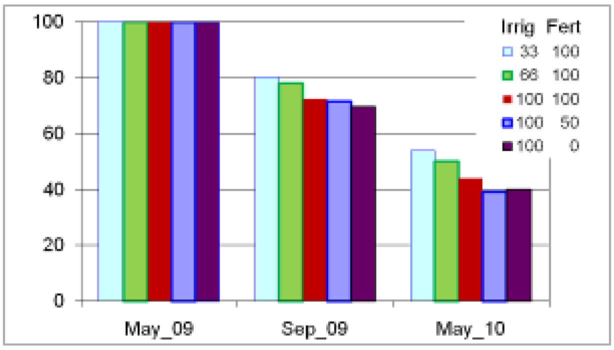

Fig. 9 Average numbers of surviving plants after summer and after winter

No statistical difference between the three doses of nitrogen was found.

Treatments that were most irrigated had higher mortality rates, but identical growths. This suggests to:

- Improve drainage and soil aeration; i.e.: remove the plastic sheets on the beds, even though this would increase weed control labour.
- Plant in sandy soils (light texture for better soil aeration and drainage).
- Reduce irrigation by at least 1/3 and use piezometers to launch irrigation (e.g. *Launch water when soil humidity level > 50 cbars*).
- Continue germplasm trials to search for varieties more adapted to cold and humid conditions.

Field results in Murcia

Five plants of each cultivar were harvested after 12 months, in March 2010. The roots, shoots (stems and twigs) and leaves were weighted separately in fresh and dry matter. Fig. 10 shows the results of dry matter yields per cultivar expressed in tons/ha assuming a planting density of 50,000 plants/ha.

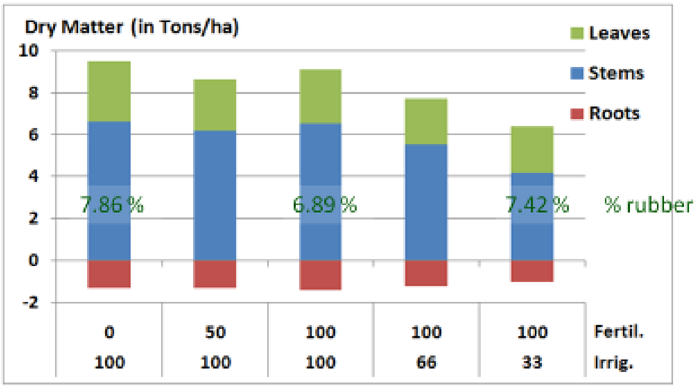

Fig. 10 Average dry matter yields at 12 months (March 2010)

Watering resulted in significantly higher yields. The climate of this region is dry and lack of water must be supplemented to perform high yields.

The average percent rubber content was 7.38 % with some plants yielding up to 9.0 % rubber. The results are high compared to Montpellier (less than 4 %). The average percentage of resin content was 7.9 %, which is not very different from Montpellier (7.0 %).

Conclusions

The best available subsets of guayule lines from Arizona germplasm have been selected.

Germplasm collection both in France and Spain were used for guayule seeds production for the project. Ten lines were selected: AZ2, AZ3, AZ5, AZ6, Cal-6, 11591, N565, 593, AZ1, and AZ 101. Germination rates of seeds produced by the project varied from 27% to 60% depending on origin, age, line and seed cleaning. Clear genetic differences were demonstrated. A method for seeds cleaning was reported by CIRAD and DLO-PRI.

Growing and overwintering of the different lines of the germplasm need to be continued. The set of 24 accessions planted in Spain and 40 accessions in Montpellier provided a good set of materials to test the genetic diversity in guayule. A cultivar trial was planted in field in year 3 (2010), with seeds of the most promising USDA lines collected from May to September 2009 in the germplasm plots in France and Spain.

A high mortality was reported in Montpellier from May 2009 to April 2010. One explanation could be that the cold weather with snow and high rainfall combined with poor soil drainage and high soil humidity on the Lavalette site near Montpellier. It can also be suspected that the plastic sheet used for weed control reduced soil aeration and increased the plant mortality. This situation resulted in poor growth (< 8 tons of dry matter·ha^{-1}) and low rubber content (< 4 % of the DM). Therefore, less than 300 kg of rubber·ha^{-1}·$year^{-1}$ could be expected.

On the opposite, guayule was well adapted to the climate and soil conditions of Murcia. The soils of the station were light and well drained. Also the lack of water due to the very dry weather could be supplemented by sufficient irrigation resulting in good yields (up to 10 tons dry matter·ha^{-1}) and good rubber content (± 7.4 % of the DM). Therefore, about 740 kg rubber·ha^{-1}·$year^{-1}$ can be expected.

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