



## Environmental, irrigation and fertilization impacts on the seed quality of guayule (*Parthenium argentatum* Gray)

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### ABSTRACT

Guayule is a perennial shrub that originates from the Chihuahuan desert. Currently stand establishment is by transplanting seedlings. In order for guayule commercialization to be more profitable, direct seeding methods need to be developed. For direct seeding to be practical factors affecting seed quality need to be identified. Guayule seed quality is highly variable. The objective of this study was to determine the seed quality of guayule (*Parthenium argentatum* Gray) grown under various field conditions in Arizona, USA, and to determine the influence of irrigation frequency and fertilization management practices on seed quality. In experiment I guayule lines AZ-2, AZ-4, AZ-R2 and 11591 were compared at four locations in Arizona (Marana, Maricopa, Yuma Mesa and Yuma Valley). In experiment II guayule lines AZ-2 and 11591 were compared under three irrigation frequencies (40%, 60% and 80%) field capacity and fertilization at low and high levels of nitrogen, at Maricopa. Germination, embryo viability, empty achene production and achene moisture content were determined for harvested achenes. In experiment I a line  $\times$  location interaction occurred for normal germination, empty achenes and achene fresh weight. Line AZ-4 had the highest germination of 59% at the Yuma Valley location. Empty achenes were the highest in Marana for line 11591 at 56%. In experiment II normal germination was affected by the line, irrigation and fertilization factors. The highest germination of 66% with line 11591, 55% at 60% irrigation and 56% at high fertilization was recorded. Empty achenes were the highest with line AZ-2 at 27%. Correlations of normal germination vs. maximum temperature, empty achenes vs. total rainfall and empty achenes vs. average wind speed were positive. Negative correlations occurred for empty achene vs. maximum temperature, normal germination vs. total rainfall and normal germination vs. average wind speed. The quality of guayule seed under both experimental conditions is severely decreased by empty achene production, which seems due to genetic variability and environmental conditions during flower bloom.

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### 1. Introduction

Guayule (*Parthenium argentatum* Gray) is a perennial shrub native to the Chihuahuan desert and occurs on the drylands of north-central Mexico (in Coahuila, Durango, Zacatecas, San Luis Potosi and Nuevo Leon) and in the Big Bend area (Presidio, Brewster and Pecos counties) of southwest Texas (Lloyd, 1911; Hammond and Polhamus, 1965; West et al., 1991). It occurs naturally at an altitude of 600–2000 m.a.s.l. and survives on sparse annual rainfall of 250–380 mm. The temperature range for survival is between –18 and 49.5 °C and plants are tolerant of high temperature but semi-dormancy is induced by low temperature of 4 °C (Thompson and Ray, 1989). The soils are well drained and calcareous with a

pH of 6–8, and have low fertility. Guayule is considered an alternative crop for production in arid and semi-arid regions of the United States (Nakayama et al., 1991), Australia (Bedane et al., 2006; Dissanayake et al., 2008) and South Africa (Milthorpe et al., 1991).

Guayule produces latex that can be processed into rubber (Artschwager, 1943) and the rubber is found to be non-allergenic when in contact with human tissues (Cornish and Siler, 1996). The latex properties include strength, elasticity and viral impermeability that make it useful in applications where durability is required and disease transmission needs to be limited, e.g. condoms, surgical gloves and catheters. Processing of guayule also produces resin for adhesives, paints and varnishes. The bagasse, which is the residual plant material that remains after latex extraction, can be used as an energy source (Campos-Lopez and Anderson, 1983). About 90% of the total biomass remains after water-based latex extraction and the residual bagasse contains compounds such as fatty

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acid triglycerides, terpenes, sesquiterpenes and waxes (Nakayama, 2005). Resin-containing bagasse can be combined with a plastic binder to produce high-density composite boards that are resistant to termite degradation (Nakayama, 2005).

Guayule seed is an achene, i.e. seed retained in dry ovary wall, with attached disk florets and subtending bracts (Lloyd, 1911). Flowering and seed-set occur under long photoperiods due to active growth when soil moisture is available, with a large flower bloom early in spring through summer, and a lesser bloom in fall (Backhaus et al., 1989). The mature fruit is dry, single seeded and produced from a double ovary of which only one develops into a seed, i.e. cypsela (Artschwager, 1943). Fertile disk florets in the center of the flowerhead, i.e. capitulum, pollinate fertile ray florets around the edge of the capitulum to produce the seeds. Each capitulum can produce up to five small achenes of about 1 mg each and numerous achenes are produced from the compound inflorescence (Hammond and Polhamus, 1965). The seed is enclosed in two seedcoats: a soft outer coat of single cell thickness, except in the vascular bundle region and a tough inner coat consisting of a one- or two-celled membrane layer of thick-walled endosperm cells (Erickson and Benedict, 1947).

Guayule seed quality is highly variable; therefore, stand establishment by direct seeding is currently not feasible to offset the cost of transplanting (Foster and Moore, 1992). Germination is affected by the natural dormancy character of the embryo and seedcoats. Freshly harvested seed quickly enters dormancy of the embryo that can last for 2 months (Hammond and Polhamus, 1965). The seedcoats contain inhibitors such as p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid that maintain dormancy for 6–12 months (Naqvi and Hanson, 1982). Guayule pollen is light and sticky, making it suitable for cross-pollination by wind and insects (Artschwager, 1943). Low percentages of seed containing fully developed embryos are produced due to poor pollination and unfavorable temperature (Benedict and Robinson, 1946) that also limit the germination potential of seed.

Guayule occurs as diploids ( $2n=2x=36$ ), triploids ( $2n=3x=54$ ), tetraploids ( $2n=4x=72$ ), and octoploids ( $2n=8x=144$ ), where numerous chromosomes are involved in meiosis (chromosome reduction division) and often results in sterility. Diploid guayule reproduces sexually, whereas triploids, tetraploids and octoploids reproduce asexually by apomixis. Apomictic plants produce sexual and asexual seeds, i.e. facultative apomixis. Diplosporous apomixis occurs in guayule, since embryo sacs are produced from diploid or unreduced megaspore mother cells. Apomixis in guayule is also considered to be pseudogamous because pollination is required for endosperm to develop from the fertilization of the central nuclei by one sperm nucleus (Estilai and Ray, 1991). Guayule also displays sporophytic self-incompatibility and many plants contain supernumerary chromosomes that complicate seed viability (Thompson and Ray, 1989).

Previous studies on improving guayule seed quality have focused on overcoming seed dormancy and have yielded positive results with osmoconditioning, polyethylene glycol, light, gibberellic acid (Chandra, 1991; Dissanayake et al., 2008), and an aqueous smoke solution treatment (Bekaardt, 2002; Bekaardt et al., 2004). Recent research (Jorge and Ray, 2005) has shown that germination can be increased by removing unfilled seeds from a seed lot. Unfilled seeds can be identified through X-ray techniques, but whether this can be used commercially to improve seed lots remains to be determined (Jorge and Ray, 2005). In Australia Bedane et al. (2006) found that maximum seed quality was achieved when seed was harvested after 329 growing degree days or about 28 days after flowering. The effects on seed quality of production location, fertility levels, and irrigation frequency have not been reported for the most recently developed guayule lines in Arizona. The objective of this study was to determine the seed quality of guayule grown under different field

conditions in Arizona, and to determine the influence of irrigation frequency and fertilization management practices on seed quality.

## 2. Materials and methods

Two experiments were conducted in Arizona, United States, in 2003 and 2004. The sites were chosen as representative of areas in Arizona where guayule is expected to be grown. Two sites in Yuma were chosen because of different soil types, with heavy clay soil at the Yuma Valley site and sandy loam soil at the Yuma Mesa site. The photoperiod for all sites was similar with 12–14 h of daylight during the seed filling period.

The line AZ-2 (PI599675, GP-9) was selected for its vigorous growth and rubber yield in 2 years, and AZ-4 (PI599677, GP-11) for its improved latex and resin content (Ray et al., 1999). Lines were developed for uniformity of plant appearance, fast growth and high latex content rubber yielding ability in four cycles of within-family selection. Seed originated from four accessions obtained from the National Center for Genetic Resources Preservation, Fort Collins, CO. Line 11591 (PI478640) is not registered, and AZ-R2 is an unreleased breeding line.

Experiment I compared lines AZ-2, AZ-4, AZ-R2 and 11591 at four locations in Arizona—Marana ( $32^{\circ}27'40''N$ ,  $111^{\circ}14'00''W$ , 601 m.a.s.l.), Maricopa ( $33^{\circ}04'07''N$ ,  $111^{\circ}58'18''W$ , 361 m.a.s.l.), Yuma Mesa ( $32^{\circ}36'43''N$ ,  $114^{\circ}38'02''W$ , 58 m.a.s.l.) and Yuma Valley ( $32^{\circ}42'45''N$ ,  $114^{\circ}42'18''W$ , 32 m.a.s.l.) (Fig. 1). A randomized complete block design with four replications was used at each location with two border rows. Each line consisted of two rows, 1 m apart and 10 m long, 36 cm between plants and 3 m between blocks. The plots at Marana, Yuma Mesa and Yuma Valley were established from transplants in May of 2002 and plots at Maricopa were established in November of 2001 from transplants. Flood irrigation was scheduled at 40% field capacity, i.e. soil moisture capacity was maintained at 40% by irrigating to 100% field capacity when moisture reached 40%. Deep neutron probes to 2 m and surface TDR (time domain reflectometer) probes to 30 cm were used to determine field moisture levels. No irrigation was applied during winter when plants were dormant.

In experiment II a split-split-split block experimental design with six replications was used. Main plots were two fertility levels (high and low), split plots were three irrigation levels (40%, 60%, and 80% of field capacity) and split-split plots were two lines (AZ-2 and 11591). Each line consisted of four rows, 1 m apart and 20 m long, 36 cm between plants and 3 m between blocks. The trial was established from transplants in April of 2002 at Maricopa comparing irrigation levels of 40%, 60%, and 80% field capacity, fertilization levels of high and low nitrogen, and lines AZ-2 and 11591. Flood irrigation was done as per experiment I to maintain each level of soil moisture. Soil nitrogen levels were determined from the 0–120 cm profile 1 month after transplanting. Average high nitrogen was at  $48.81 \text{ kg ha}^{-1}$  and low nitrogen was at  $16.35 \text{ kg ha}^{-1}$ . High fertility plots were established following 4 years of continuous alfalfa production and low fertility plots followed cotton and unfertilized sudangrass to mine available nitrogen from the soil. The sudangrass exhibited nitrogen deficiency symptoms before the experiment was started. Weather data of temperature, rainfall and wind speed for the trial period from 2003 to 2004 was obtained from The Arizona Meteorological Network website (2005).

Dried inflorescences were harvested by hand from plots during summer of 2003 and 2004. One to 2 weeks after harvest samples were run through a belt thresher and blown in an air column to remove chaff. No further selection was done to remove achenes based on color or other criteria. Standard germination tests were adapted from AOSA (2002) and performed on sample units of 50 achenes per treatment per replication since each treatment was

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