

DISSERTATION

Seed Quality of Guayule (*Parthenium argentatum* Gray)

Submitted by

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In partial fulfillment of the requirements
for the Degree of Doctor of Philosophy
Colorado State University
Fort Collins, Colorado
Spring 2006

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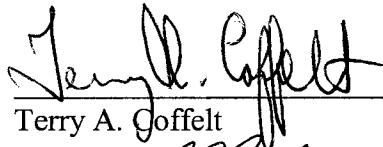
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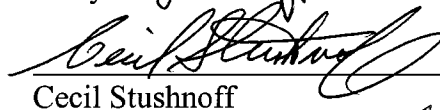
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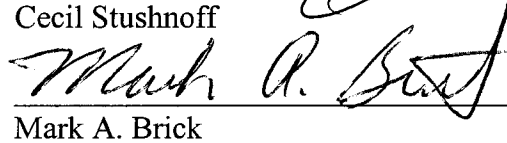
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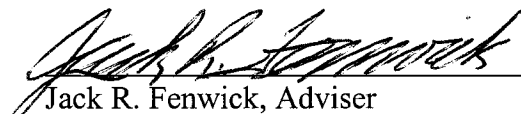
WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY **CLAUDE RON BEKAARDT** ENTITLED **SEED QUALITY OF GUAYULE (*Parthenium argentatum* GRAY)** BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

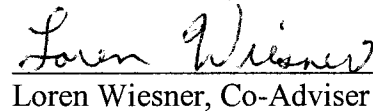
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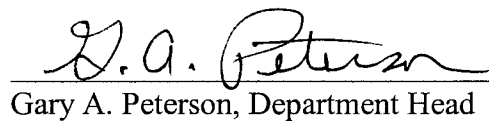

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ABSTRACT OF DISSERTATION

Seed Quality of Guayule (*Parthenium argentatum* Gray)

The germination potential of guayule seed is considered too poor for direct seeding. Relating seed quality to field conditions could provide information on suitable locations and management to improve seed quality. The study objective was to determine seed quality of guayule grown under different field conditions in Arizona. Two trials were evaluated: experiment I, compared lines AZ-2, AZ-4, AZ-R2, and 11591 at locations Marana, Maricopa, Yuma Mesa, and Yuma Valley, and experiment II compared lines AZ-2, and 11591 under integrated irrigation of 40%, 60%, and 80% field capacity, and fertilization of high and low nitrogen. Seed germination, empty achene production, viability of ungerminated seeds and moisture content was determined. In experiment I, a line x location interaction occurred for normal germination, empty achenes, and achene wet weight. Yuma Valley location and line AZ-4 had the highest seed germination at 59%. Empty achenes were higher in Marana for 11591 at 56%. Achene wet weight was higher at 0.041g/50 achenes in Yuma Mesa with 11591 and in Yuma Valley with AZ-R2. In Experiment II, line, irrigation, and fertilization were significant for normal seed germination. Only line was significant for empty achene production. Line x fertilization and irrigation x fertilization interactions were significant for achene wet weight. Seed germination was higher with line 11591 at 66%, 55% at 60% irrigation, and 56% at high fertilization. Empty achenes were higher with AZ-2 at 27%. Achene wet weight was at 0.033g/50 achenes, and higher for 11591 at high and low fertilization levels, and higher at 40% irrigation and high fertilization, and at 60% irrigation and low fertilization. Experiment I had overall seed germination at 44%, and experiment II was at 39%. Empty

achene production was at 36% in experiment I, and 31% in experiment II. The quality of guayule seed under both trial conditions were severely decreased by empty achene production. There is not any indication that soil moisture and nutrient applications improves the seed germination potential. The main reason for empty achene production seems due to the genetic variability of guayule seed production, and related to the environmental conditions of high temperature, rainfall and winds during flower bloom at the trial sites.

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DEDICATION

I dedicate my achievements to the memory of my mother Mary (Spandiel) Bekaardt, and in honour of my father Dawid Bekaardt; To the love of my wife Mathilda, and my sisters Linda, Mary and Denise, and my brother Dave; To the heritage of our promising future generation: my son Gryphon, Nadine and Leonardine, Ronaldo and Marion, Dillon and Zane; To the hope and steadfast belief of my family and friends; And ultimately, to the glory of God and His infinite grace.

TABLE OF CONTENTS

| | <u>Page</u> |
|-------------------------------------|-------------|
| ABSTRACT | iii |
| ACKNOWLEDGEMENT | v |
| DEDICATION | vi |
| LIST OF FIGURES | viii |
| LIST OF TABLES | x |
| LITERATURE REVIEW | 1 |
| Background | 1 |
| Origin | 3 |
| Classification and morphology | 6 |
| Cultivation practices | 11 |
| Crop management | 16 |
| Harvesting practices | 18 |
| Plant breeding | 19 |
| Overview | 21 |
| SEED QUALITY EVALUATION | 23 |
| Introduction | 23 |
| Materials and methods | 26 |
| Results | 30 |
| Discussion | 49 |
| Conclusion | 51 |
| LITERATURE CITED | 53 |

LIST OF FIGURES

| | <u>Page</u> |
|---|-------------|
| Fig. 1. Distribution of native guayule in Mexico and Texas | 4 |
| Fig. 2. Potential areas for guayule cultivation in the United States | 5 |
| Fig. 3. Guayule branch with leaves and inflorescences and with achene-complex insets | 7 |
| Fig. 4. Guayule inflorescence | 8 |
| Fig. 5. Achene-complex in guayule | 10 |
| Fig. 6. Branch of guayule inflorescence with flowers in various stages of development | 13 |
| Fig. 7. Distribution of Arizona weather stations indicating trial locations at Marana, Maricopa, Yuma Mesa and Yuma Valley | 28 |
| Fig. 8. Regression correlation of Normal Germination vs. Empty Achenes | 35 |
| Fig. 9. Regression correlation of Normal Germination vs. Achene Wet Weight | 36 |
| Fig. 10. Regression correlation of Normal Germination vs. Empty Achenes | 41 |
| Fig. 11. Regression correlation of Normal Germination vs. Achene Wet Weight | 42 |
| Fig. 12. Minimum monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004) | 43 |
| Fig. 13. Maximum monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004) | 44 |
| Fig. 14. Average monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004) | 45 |
| Fig. 15. Total monthly rainfall at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004) | 47 |

Fig. 16. Average monthly wind speed at four locations (Marana, Maricopa, Yuma Mesa,
and Yuma Valley) over the trial period (January 2003 to December 2004)
.....48

LIST OF TABLES

| | <u>Page</u> |
|--|-------------|
| Table 1. Analysis of variance for Normal Germination, Empty Achenes, and Achene Wet Weight of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona | 31 |
| Table 2. Normal Germination (%) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona | 32 |
| Table 3. Empty Achenes (%) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona | 33 |
| Table 4. Achene Wet Weight (g/50 achenes) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona | 34 |
| Table 5. Analysis of variance for Normal Germination, Empty Achenes, and Achene Wet Weight of guayule lines AZ-2 and 11591, from location at Maricopa, in Arizona | 38 |
| Table 6. Normal Germination (%), Empty Achenes (g/50 achenes) and Achene Wet Weight (g/50 achenes) of guayule lines AZ-2 and 11591, under 40%, 60% and 80% field capacity irrigation, and at high and low fertilization levels | 39 |
| Table 7. Interaction of guayule lines AZ-2 and 11591, with high and low fertilization levels with respect to Achene Wet Weight (g/50 achenes) | 40 |

LITERATURE REVIEW

Background

Parthenium argentatum (Gray, Asteraceae), commonly known as guayule, pronounced *why-you-lee*, has been a potential domestic source of natural rubber for the United States since its discovery in 1802 (Artschwager, 1943; Hammond and Polhamus, 1965). The use of rubber in the manufacturing of vehicle tires has driven the rubber market since Charles Goodyear was able to make it unresponsive to temperature change (Lloyd, 1911; Bonner, 1991). Guayule and *Hevea brasiliensis* (Meuller-Agroviensis, Euphorbiaceae) rubber were compared for characteristics of yield, strength and durability to meet the demands of the motor vehicle industry. *Hevea* development benefited immensely from research efforts of the Rubber Research Institute in Malaysia in 1925, which developed the crop from wild stands and provided a uniform and stable source of rubber (Bonner, 1991). Guayule research and development however, was limited by sporadic research efforts that resulted in non-uniform stands and lower yields when compared to *Hevea*.

By 1992, about 500,000 people in the United States showed allergic responses when in contact with *Hevea* rubber (Hamann, 1993). Proteins in *Hevea* rubber lead to the risk of damage to the nervous system in cases of severe allergic reactions, i.e. Type I allergy (Cornish and Siler, 1996). Guayule rubber proteins was non-allergenic in contact

with human tissues (Cornish and Siler, 1996), which motivated guayule rubber research for development of products for use in the medical and health-care industry. Guayule latex properties include strength, elasticity and viral impermeability that make it useful in applications where durability is required and disease transmission limited, e.g. condoms, surgical gloves and catheters. Processing of guayule also produces resin for adhesives, paints and varnishes, and the bagasse, which is the residual plant material after latex extraction, can be used as an energy source (Campos-Lopez and Anderson, 1983). About 10% of the total biomass is used in water-based latex extraction and the bagasse residual contains compounds such as fatty acid triglycerides, terpenes, sesquiterpenes, and waxes (Nakayama, 2005). Resin-containing bagasse can be combined with a plastic binder to make high-density composite boards that are resistant to termite degradation. The plant is useful in numerous applications: as a soil stabilizer, as compost, wax can be extracted, and the flowers can be used in fresh and dried arrangements.

The first utilization of guayule rubber, other than by natives, began in 1888 (Lloyd, 1911), but initial experimentation with guayule rubber only began in 1902 when the high price of imported rubber was recognized as a potential limitation of rubber supplies to the United States (Bonner, 1991). The utilization of guayule rubber was restricted by the Mexican Revolution in 1912, the sporadic wars after 1941, the Great Depression, the freezing of developmental funds, and the resulting recession that forced the liquidation of rubber companies (Hammond and Polhamus, 1965). Despite extensive cultivation and intensive research, guayule rubber was not commercially produced anywhere in the world after 1962 and harvesting from wild stands in Mexico had stopped. The current interest in guayule started in the 1970's with the rise in oil prices

that resulted in the search for local resources of natural products. Funding for investigation into guayule was provided by the National Science Foundation in 1976 and research on agronomy, breeding, rubber evaluation, economics, and technology assessment was initiated by the United States Department of Agriculture in 1980 (Stewart *et al.*, 1986).

Origin

Guayule is a perennial shrub that originates from the Chihuahua desert, Mexico, and occurs on the drylands of northcentral Mexico, and in south and southwest Texas, between 22° 30' and 31° 30' N longitude and 105° and 100° W latitude (Fig. 1) (Lloyd, 1911; Hammond and Polhamus, 1965, West *et al.*, 1991). It occurs naturally on slopes at an altitude of 600 to 2000 m above sea-level and survives on annual rainfall of 250 to 380 mm. The soils are well-drained and calcareous with a pH of 6 to 8 and the habitat temperature ranges from -18 to 49.5 °C. Guayule can survive under high temperatures, but temperature below 4 °C induces semi-dormancy and prolonged freezing causes death (Thompson and Ray, 1989). Potential areas for guayule cultivation in the United States are regions of California, Arizona, New Mexico, and Texas (Fig. 2) (Nakayama *et al.*, 1991).

Regions in South Africa where guayule production may be possible are east of the Kalahari, the Eastern Cape, Free State, North-West and the Northern Province (Smit, 1989; Milthorpe *et al.*, 1991). Field evaluations in Upington, Northern Cape, and Bethulie, Free State, indicated the survival potential and biomass production capability of guayule (Bekaardt, 2002; Bekaardt *et al.*, 2005a).

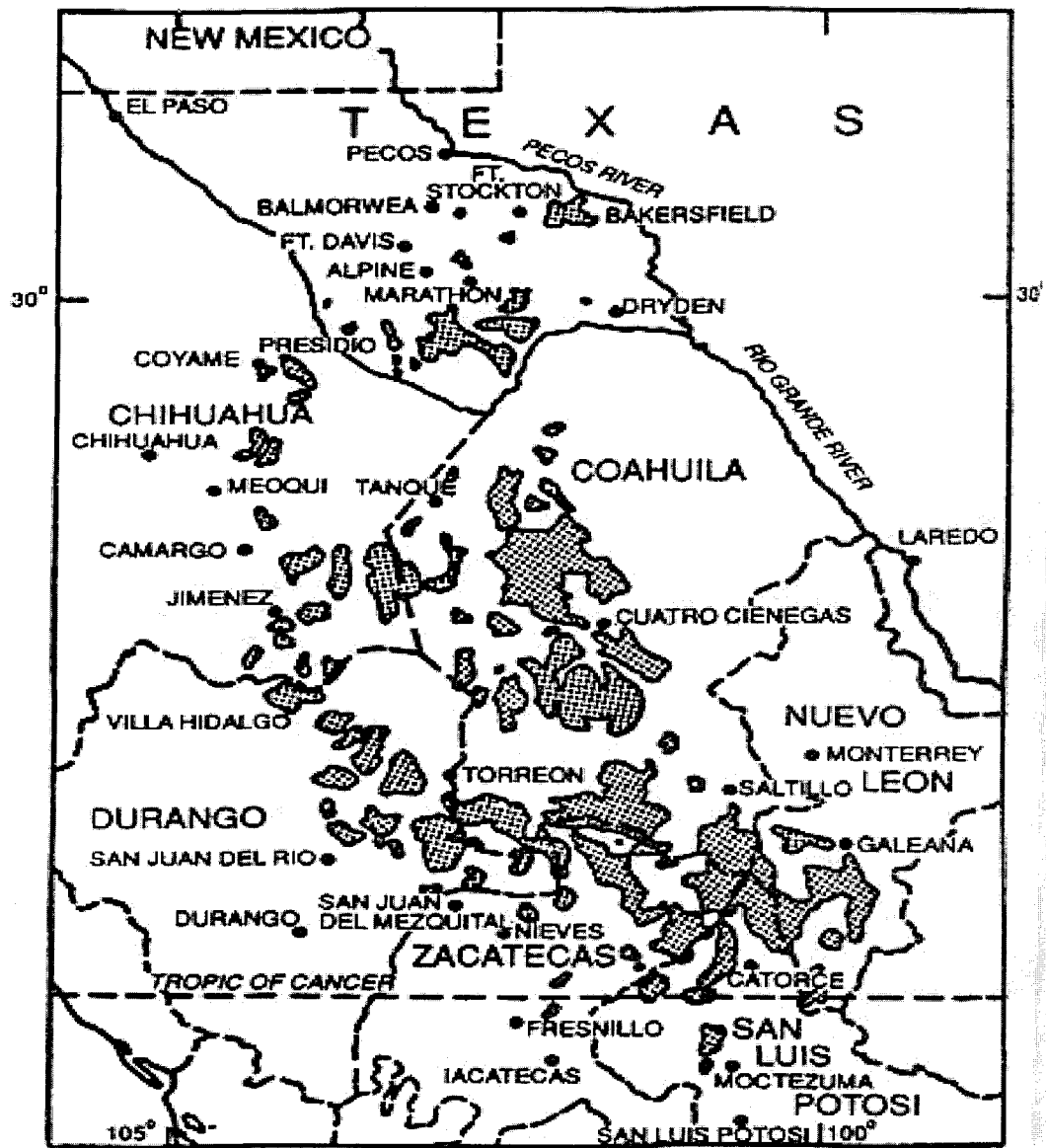


Fig. 1. Distribution of native guayule in Mexico and Texas (After Jenkins, 1946) (West *et al.*, 1991).



Fig. 2. Potential areas for guayule cultivation in the United States (below solid line) (Nakayama *et al.*, 1991).

Classification and morphology

Parthenium argentatum (Gray) is a member of the Asteraceae family and the Heliantheae tribe (Lloyd, 1911). Guayule shares similar structures of inflorescence (numerous small male and female flowers on a common receptacle) and achene (seed retained in a dry ovary wall) production with sunflowers (*Helianthus sp.*) and dandelions (*Taraxacum sp.*).

Guayule grows to a height of about 1m, has numerous branches with gray-green leaves and yellow inflorescences (Fig. 3) (Hammond and Polhamus, 1965). The leaves are partially deciduous, since the lower leaves shrivel up and shed during winter drought. T-shaped trichomes cover the dorsal and ventral surfaces of the lobed leaves. The branches end in the formation of inflorescences. Branches lignify (become woody due to lignin deposition) during the first year of growth. The taproot gives rise to lateral, fibrous roots with intricate branches, allowing the uptake of water in deep and upper soil region.

Latex is found in all plant structures, with the greatest concentration in the bark of stems, roots, and branches (Fangmeier *et al.*, 1984). In young plants latex occurs mainly in the primary cortex, vascular rays, parenchymous cells, and pith cells, but in mature plants it occurs in the vascular rays of the xylem and phloem (Artschwager, 1943).

The inflorescence is a compound, one-sided cyme, with flowers contained in heads on common receptacles (Fig. 4) (Hammond and Polhamus, 1965). Five ray florets that contain the fertile pistils, each with two attached sterile disk florets, occur on the periphery of the head, while disk-florets that contain the fertile stamens occur in the center. Flowering is continuous throughout the growing season, i.e. indeterminant, and

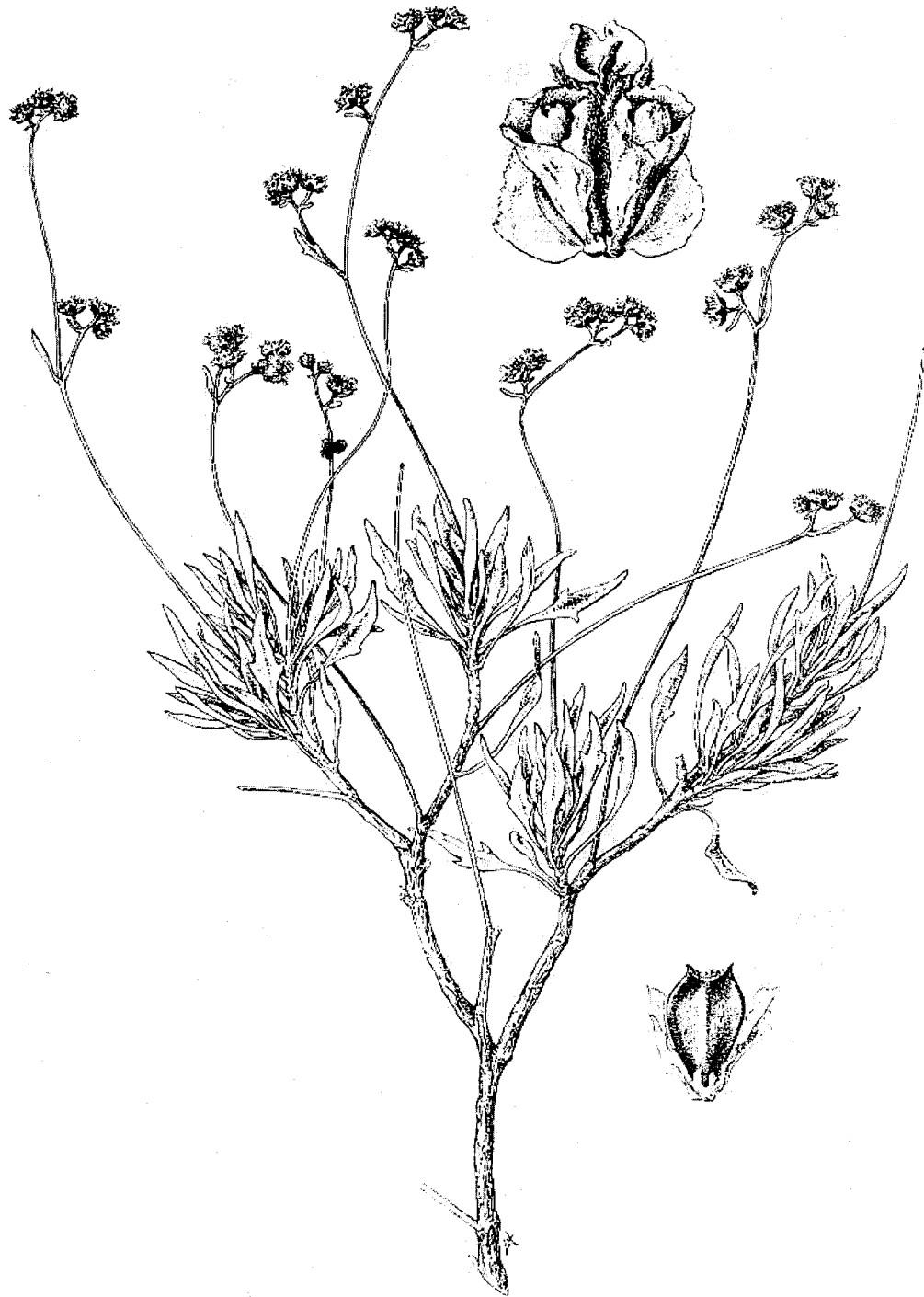


Fig. 3. Guayule branch with leaves and inflorescences and with achene-complex insets (Hammond and Polhamus, 1965).

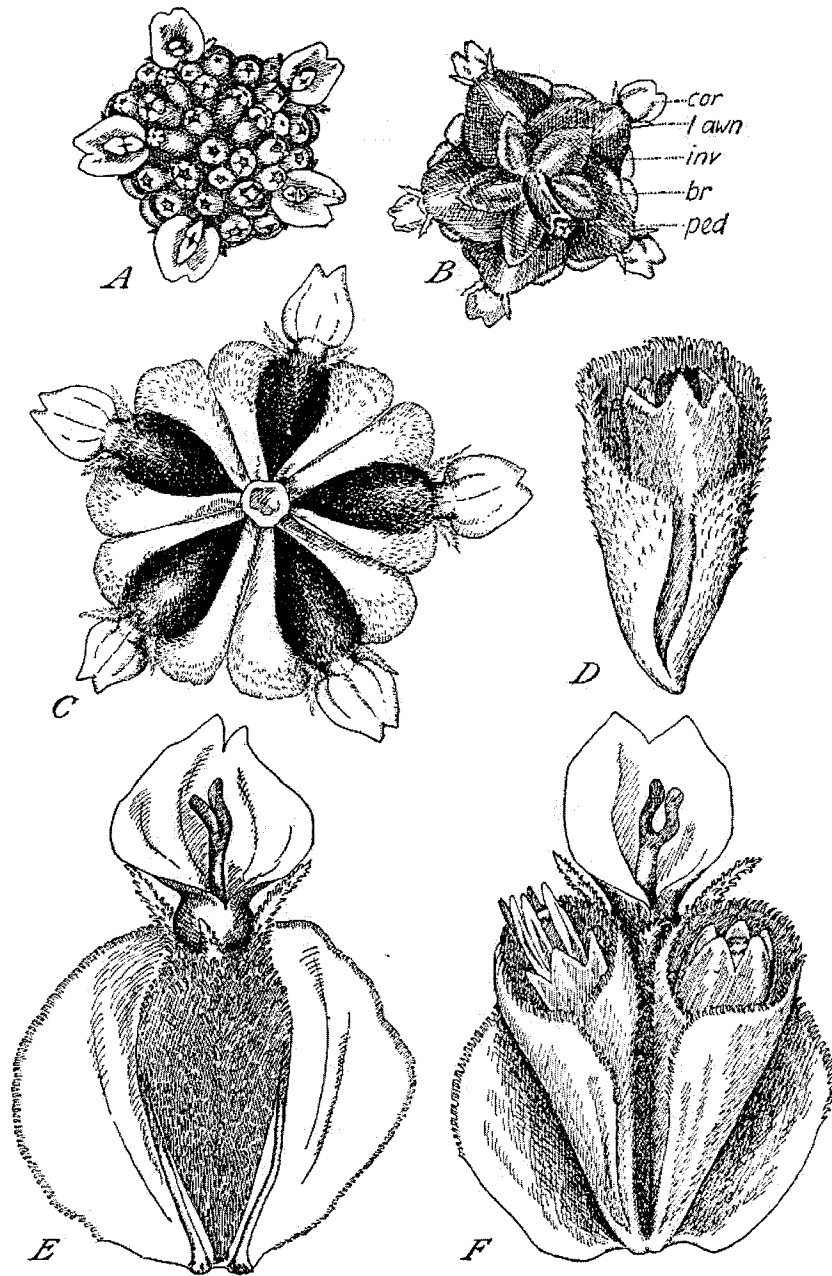


Fig. 4. Guayule inflorescence. *A*, Surface view of flower head showing 5 ray (female) and 24 disk (male) florets. *B*, Rear view of flower head; *cor*, lobe of corolla; *l awn*, lateral awn; *inv*, involucre leaf; *br*, bract of ray flower; *ped*, pedicel. *C*, Enlarged rear view of mature head with involucre bracts removed to show outlines of achenes with attached sterile flowers, ray corollas and pappus. *D*, Disk flower with enclosed bract. *E*, Enlarged view of ray flower and bract without its disk flowers. *F*, Ray flower with 2 adnate disk flowers (Artschwager, 1943).

results from active growth under favorable moisture conditions, habitually during summer. Pollination of the flowers is by the wind and insects such as lady beetles (*Scymnus* sp.), lygus bugs (*Lygus* spp.), honeybees (*Apis* spp.) and bumblebees (*Bombus* spp.). The pollen grains are light and the outer surfaces are sticky and spiny so that they can adhere to insects and be transferred to the stigma (Hammond and Polhamus, 1965).

Guayule fruit is an achene (Fig. 5) that weighs less than 1 mg/achene and is shed from the plant as a unit called the achene-complex, with the two sterile disk-florets, subtending bract, persistent ligule, and the two-lobed stigma attached (Lloyd, 1911; Hammond and Polhamus, 1965). The mature fruit is dry, and is produced from a double ovary, of which only one develops into a seed, i.e. cypsela. Numerous small achenes are produced from the compound inflorescences. A fully developed achene contains an embryo that is enclosed in two seedcoats: (1) a soft outer coat of single cell thickness, except in the vascular bundle region, and (2) a tough inner coat consisting of a one- or two-celled membrane layer of thick-walled endosperm cells (Erickson and Benedict, 1947). The seedcoats contain inhibitors such as p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid, and ferulic acid that delay germination (Naqvi and Hanson, 1982).

Guayule accessions consist of diploids ($2n=2x=36$), triploids ($2n=3x=54$), tetraploids ($2n=4x=72$) and octoploids ($2n=8x=144$) (Thompson and Ray, 1989). Polyploids generally reproduce by facultative apomixis i.e. sexually and asexually, and diploids mostly sexually. Sporophytic self-incompatibility occurs in guayule that limits successful fertilization opportunities, and supernumerary chromosomes exist that lead to complications in meiosis and result in sterility.

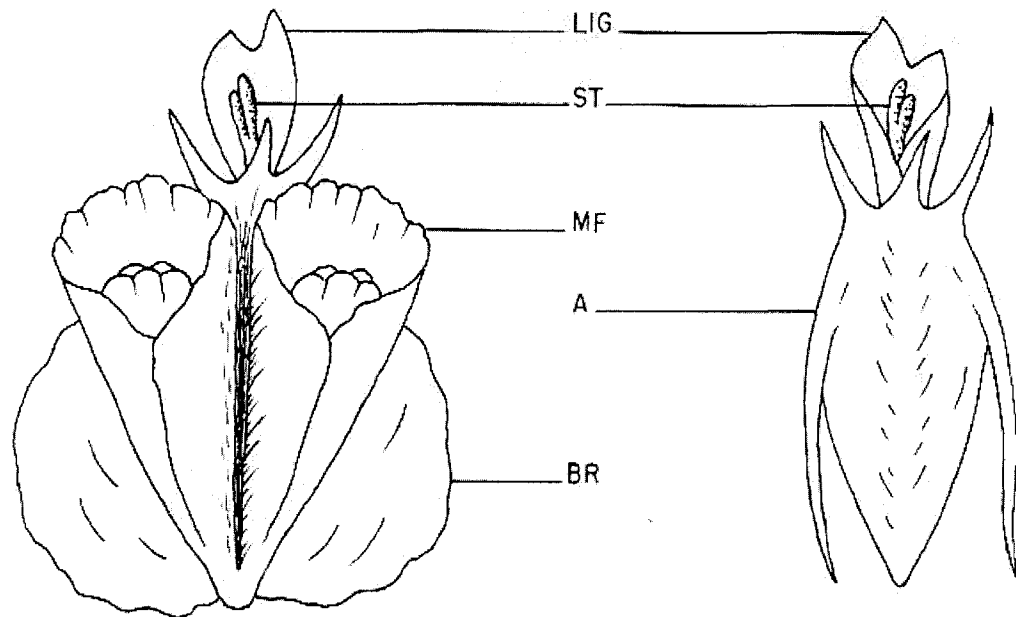


Fig. 5. Achene-complex in guayule. LIG, persistent ligule (corolla); ST, persistent two-lobed stigma; MF, attached disk (male) floret; A, achene; BR, subtending bract (Lloyd, 1911).

Cultivation practices

Guayule establishment results from the germination of seed and the development of seedlings in the nursery. Seed germination is affected by the dormancy character of the embryo and dormancy due to inhibitors in the seedcoats. Freshly harvested seed quickly enters dormancy of the embryo that can last for 2 months (Hammond and Polhamus, 1965), whereas the seedcoat induced dormancy can be maintained for 6 to 12 months (Naqvi and Hanson, 1982). Low percentages of achenes containing fully developed embryos also limit the germination potential of seed (Benedict, 1950; Benedict and Robinson, 1946). Seed germination has been improved with the use of gibberellic acid in combination with planting depth, light (Hammond, 1959), gibberellic acid and an aqueous smoke solution (Bekaardt *et al.*, 2004). These techniques are sufficient for seed germination to develop nursery stock that can be transplanted to the field, and is the most reliable method of guayule stand establishment (Foster and Coffelt, 2005). When large fields are to be established, direct seeding is more economical, because the labor cost involved in raising seedlings in the nursery is omitted (Foster and Moore, 1992). The cost of establishment by direct seeding is approximately US\$ 400/ha versus US\$ 900 to 1200/ha by means of transplanting (Bucks *et al.*, 1986). Direct seeding has been successful in research plots in Texas, New Mexico, and Arizona, using seed conditioning techniques and precision planting (Foster and Coffelt, 2005), but large field plantings by direct seeding has not been successful due to poor seed quality.

Seeds are small and produced in large numbers per plant (Stewart and Henderson, 1986) since the flowers that bear the seeds are borne in clusters, with five seeds formed in each flower. The seeds have a long period of viability due to the dormancy characteristics

of the embryo and seedcoats (Hammond and Polhamus, 1965) that allow them to survive during periods of drought when conditions for growth are unfavorable. Seeds have a low germination potential and a high post-germination mortality (Stewart and Henderson, 1986) probably due to poor seedling vigor (Fig. 6).

Retonos, or shoot propagation, are a form of vegetative reproduction that occur when branches and roots become separated from the mother-plant through destruction by feeding or grazing animals, or by soil erosion and give rise to new plants. Lateral branches that come in contact with the soil produce adventitious roots that obtain nutrients from the soil to sustain the branch sufficiently to eventually separate completely from the mother-plant. Roots that are separated from the mother-plant can also produce adventitious shoots to produce an independent individual plant (Lloyd, 1911).

Nursery reproduction by means of seed, cuttings and tissue culture provide the opportunity for experimental research procedures on propagation. Seed propagation is presently the most cumbersome process because it involves a process of cleaning and threshing. Harvested seed contain tightly bound disk-florets and other flower material that requires a mechanism of separation that can only be achieved successfully by adjustments to a standard thresher. The thresher needs to have a close rubbing belt-action that will break the flower clusters from each other and release the achene. It also needs a screen (0.21-1.27mm slots) that is small enough to allow most floral debris to be removed (Hammond and Polhamus, 1965).

Furthermore, the embryo and seedcoat dormancies are dependent on environmental conditions such that dormancy will only be released when environmental conditions are favorable for growth. The seedcoats can be adequately degraded over time

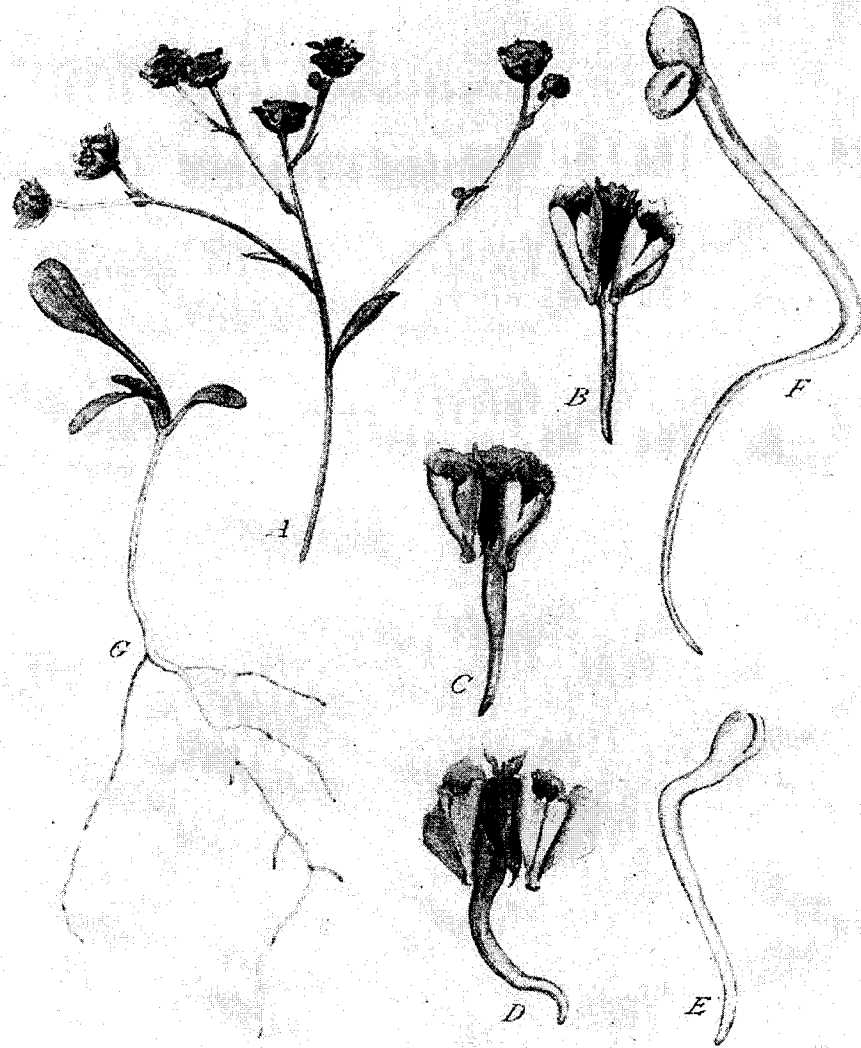


Fig. 6. *A*, Branch of guayule inflorescence with flowers in various stages of development. *B-D*, germinating seed. *E-F*, young seedlings. *G*, older seedling (Artschwager, 1943).

by microbial activity to allow moisture and oxygen penetration to activate the processes of germination. Germination is found to be spontaneous and extremely erratic since these activities are orchestrated by natural processes. Direct seeding has been largely rejected as an effective means of stand establishment due to the dormancy characteristics of the seed. Seed germination in seed trays and with treatment solutions (Hammond, 1959) have turned out to give the most satisfactory results.

Seedling survival tends to be very low and could be mainly due to naturally poor seedling growth after germination. In the nursery environment, seedlings can be maintained in a growth medium of sand, peat-moss and polystyrene chips (2:1:1) that provides good water filtration and aeration (Bekaardt *et al.*, 2004).

Nursery grown seedlings need a source of nutrients, since they are grown in a sterile growth medium (Bekaardt, 2002). Nutrients can be supplied from a general, water-soluble nutrient mix, either through the irrigation water supply or by means of a watering can. Three-month old seedlings should be strong enough to be removed from the germination area to a 50 % shade area, where water supply is diminished and nutrient supply is removed. This procedure hardens the plants in preparation for field planting about one week later. Once the seedlings have been transplanted they should be supplied with adequate water from short frequency spray irrigation. Seedlings are prone to aphid and snail feeding, but these can be easily controlled by selective aphid spraying and snail bait.

Vegetative propagation by means of cuttings primarily involves taking ± 5 cm terminal cuttings, treating it with a rooting hormone such as indole butyric acid (Hammond and Polhamus, 1965), and planting it in a growth medium. The rooting

potential of guayule was demonstrated with different concentrations of indole butyric acid, naphthalene acetamide and naphthalene acetic acid (Bekaardt *et al.*, 2005b). Though extensive investigations were done by numerous researchers, no consistent results could yet be achieved. This method of propagation seems largely impractical for cultivation practices (Erickson and Smith, 1947), but does have merit when elite lines are identified that can be cloned.

Tissue culture techniques have been developed that successfully produce plantlets from apical buds (Smith, 1983). Benzyladenine (1 mg/L) was found to stimulate shoot growth, whereas indole butyric acid (0.5 mg/L) initiated root formation from these shoots. This method of asexual reproduction provides numerous plantlets that are genetically identical and has great potential where desired traits such as lush growth and increased rubber or resin production have been identified.

Guayule transplanting is done in early March to mid April in Australia after seasonal rain to make use of moisture retained in the soil (Stewart and Henderson, 1986). For dryland crop management, irrigation should be applied weekly after initial transplanting for about one month until the plants are successfully rooted and are able to uptake water from the soil. Plants are commonly placed in rows of 1 to 1.2 m apart and with 30 to 50 cm between plants. Row spacing is mainly determined by available machinery for planting, weed control and harvesting, while spacing between plants is governed by the growth form of the selected line, i.e. whether it is more upright or spreading, and whether seed will be collected manually or mechanically.

Crop maintenance

Land preparation begins with the selection of the most suitable site for guayule establishment. It follows naturally that the extent of land selection is directly related to the original condition of the soil. It is thus important to be conscious of the native habitat of wild guayule populations, which thrive on calcareous, loose, permeable, well drained, and coarse to medium textured soils in the pH range of 6 to 8 (Hammond and Polhamus, 1965). Transplanted seedlings are prone to initial drowning and require a well-drained soil for optimal root penetration during initial establishment, which could be attained by deep plowing (15 to 20 cm).

Fertilization is not considered a crucial crop management tool since guayule has a low nutrient requirement and is specifically implemented in areas with poor soil types (Hammond and Polhamus, 1965). An increase in available nutrients does have the potential to increase biomass production on extremely poor soils. Nitrogen at 50 kg/ha and phosphorous at 20 kg/ha are suggested for poor soils in Australia (Ferraris, 1986). Field experiments have yet to prove that related costs of soil fertilization can be justified by the amounts of latex and resin produced. Maximum guayule production may require 1000 to 1300 mm/pa of applied water, consisting of irrigation and rainfall (Foster and Coffelt, 2005).

Weed infestation in guayule fields is extremely important during the establishment of the young plant. It is easily overrun by weeds in the early stages of establishment due to its slow growth rate. A broad-spectrum herbicide would be useful for weed control, but currently no herbicides are registered for use in all areas. Pendimethalin [*N*-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine] is registered for

special local needs in Arizona and is considered safe for preemergence weed control in transplanted guayule, and controls 68% of weeds at an application rate of 0.6 to 1.1 kg/ha (Foster and Coffelt, 2005). A number of commercial herbicides such as oxyfluorfen (Goal), oryzaline (Surflan), and DCPA (Dacthal) have been tested in trial plantings with positive results (Whitworth, 1981; Milthorpe, 1984). The best approach to weed control is governed by good agricultural practices, since no broad-spectrum weed control agent has yet been registered for general use in guayule. Simple weed control can be done by cultivating weeds between rows and hand-weeding between plants, thus ensuring that the crop is not overgrown by or adversely affected by weed competition.

Soil borne diseases in guayule plantings are mostly due to poor water filtration and can be remedied by selecting soil with good drainage. Soil-borne diseases usually cause root rot that terminates the plant's ability to draw water and nutrients from the soil (Hammond and Polhamus, 1965). Related diseases include wilting of tops caused by the soil fungus *Verticillium dahliae* Kleb., which attacks the roots and interferes with water supply. *Phymatotrichum omnivorum* (Shear) Duggar., causes root rot that leads to sudden wilting, drying and curling of the leaves. *Sclerotium bataticola* Taub., causes charcoal or crown rot that appears as black and dark brown sunken lesions and dying of the tops (Mihail *et al.*, 1991). Seedling root rot is caused by *Phythium ultimum* Trow., which attacks the taproot or root crowns and causes pinkish or reddish color of woody cylinders at lesions. Insect pests on cultivated guayule include lygus bugs (*Lygus* spp.) that feed on developing seeds and succulent growing tips, thrips (*Frankliniella* spp.) and aphids (*Aphis gossypii*, *Myzus persicae* and *Anuraphis helichrysi*) that cause leaf curling and distortion, and grasshoppers (*Melanoplus* spp.) that feed on leaves.

Harvesting practices

Seed harvesting is dependent on prevailing environmental conditions, since flowering with resultant seed-set can occur continuously over the growing season if soil moisture is adequate to maintain vegetative growth (Hammond and Polhamus, 1965). The guayule seeds are borne in the flowerheads and loosen from the cluster when mature. A number of mechanical seed harvesters have been used by the Intercontinental Rubber Company that functions on a vacuum system for seed collection (Hammond and Polhamus, 1965). Improvements on this basic design incorporated a pressure and suction blower for seed collection (Stewart and Henderson, 1986). Other designs include a harvester with a vibrator to dislodge seed and a blower to move seed to suction inlets (Tysdal, 1981). Coates (1985) developed a seed harvester that works with a rotating brush to dislodge seed and later improved on the design by using multiple impact and scraping to dislodge seed, and included a seed collecting surface (Coates, 1986).

Latex harvesting of guayule involves two approaches: (1) complete removal of the plant (branches and roots) after 5 to 6 years of growth, as employed in the Emergency Rubber Project and (2) ratoon cropping (clipping of the branches 5 to 10cm above ground level) after two years of growth (Lloyd, 1911), and then complete harvesting of the regrown shrub after 6 to 7 years (Hunter *et al.*, 1959). Guayule shrub harvesting equipment first used by the Intercontinental Rubber Company focused on complete removal of the plant by digging using a two-row digger mounted behind a tractor (Hammond and Polhamus, 1965). Further development of equipment incorporated the idea of clipping (pollarding), with different cutting tools that were evaluated by Coates (1986) and investigation on the regrowth in response to clipping (Garrot and Ray, 1983).

Harvesting time takes into consideration the growth stage of the plant, having predominant stages of active growth in summer and a dormant period in winter. The method of harvesting to be employed also determines the scheduling of harvesting. Plants that will be re-grown after harvesting require more care against damage than plants destined for complete harvesting, as considered by Lloyd (1911). Plants destined for regrowth should thus be harvested during the dormancy stage after active growth has stopped to diminish the risk of infection, since the plant has already stored its biochemical products and has already halted its metabolic activity in preparation for the resting stage. Special consideration should be given to the chilling-requirement of the plant in its production of latex (Bonner, 1943; Benedict, 1950). Though it has not been proven scientifically, the production of latex could presumably increase the ability of the plant to withstand extreme cold climatic conditions. Thus during the winter dormancy stage, the plant has already accumulated its greatest potential of latex for the purpose of surviving adversely cold weather.

Plant breeding

Guayule breeding draws on the existing variability found in the available germplasm. Generally polyploids reproduce by facultative apomixis and diploids by sexual reproduction that increases the variability in the germplasm. Attention has been focused on improving rubber yields per unit area, rubber quality, regrowth after clipping, processing quality of the shrub, disease and insect resistance, and reducing postharvest rubber degradation (Thompson and Ray, 1989). Rubber yield may be improved by increasing biomass and/or rubber content because it can be expressed as a product of rubber content (percent rubber) and biomass (dry weight per unit area) (Estilai and Ray,

1991). By using minimum selection thresholds (plant selection based on minimum value of a single trait) for both biomass and rubber content, the negative correlation between biomass production and rubber content has been overcome to improve the crop (Ray, 1993). Additional characteristics of the plant that have received attention are increased resin and biomass production, seed size and yield per plant, and tolerance to environmental stresses and their interaction with rubber production.

The development of new lines has been achieved mostly by single-plant selections from apomictic polyploid populations, whereas hybridization (cross between selected plants) of polyploids and interspecific hybridization (cross with related species) has been used on a limited scale. Crop improvement has been accomplished with newer lines yielding up to 250% more rubber than lines developed in the 1940's and 1950's (Ray *et al.*, 2005). Breeding with diploid plants has been favored by the use of applicable standard breeding techniques. Modified recurrent selection improved yields whereas mass selection provided *Verticillium* tolerant lines. *Verticillium* wilt is a common root disease of guayule that has been addressed through selection for tolerance (Thompson and Ray, 1989; Estilai and Ray, 1991). Diploids have been crossed with apomictic polyploids to produce new genetic combinations and high yielding polyploids have been crossed with diploids resulting in greater phenotypic expression (Thompson and Ray, 1989; Estilai and Ray, 1991).

Plant breeding has made specific selections and new lines were developed with increased biomass, latex and resin production. These are AZ-1 with improved rubber yield at three years, AZ-2 and AZ-3 with vigorous growth and rubber yield within two years, AZ-4 and AZ-5 with improved rubber and resin content, and AZ-6 with improved

rubber yield and plant uniformity [Released by USDA-ARS June 18, 1997. Crop Sci. 39:300 (1999)]. These six germplasm lines were developed in cooperation with The University of Arizona for uniformity of plant appearance, fast growth, high rubber content, and rubber yielding ability in four cycles of within-family selection in field plots at The University of Arizona, Maricopa Agricultural Center. Seed originated from four accessions obtained from the National Centre for Genetic Resources Preservation. Evaluation of genotype, environment, and genotype x environment effects indicates environment (50%), plant age (16%) and line (10%) as significant factors that determine variation in plant growth, biomass production and latex content of guayule (Coffelt *et al.*, 2005). The available germplasm exhibits extreme variability within and between lines for morphological traits such as height, width, and biomass; and chemical constituents such as rubber and resin contents. The measured variation is due to facultative apomixis that cause genetic variation among the progeny, and to a greater extent is due to the environment (Ray *et al.*, 2005).

Overview

Guayule is a plant of the Chihuahuan desert that has experienced years of extensive, though intermittent, investigations since 1902. Among domestic sources of natural rubber in the United States of America, guayule was found useful in producing rubber of comparable quality and strength to *Hevea*, and also distinguished itself in producing rubber with non-allergenic properties and viral impermeability. As a dryland crop guayule has the potential to be incorporated into agricultural systems in areas that cannot be utilized for existing crop production due to a lack of sufficient rainfall and poor soils. There is great potential to establish guayule farming activities on marginal lands

since the plant does not require intensive soil fertilization and is capable of surviving under extreme heat and cold when the plant is mature. Technology is currently available on plant physiology, genetics, breeding, propagation, cultivation and agronomy, harvesting and processing to successfully utilize guayule as an alternative crop. The development of products and markets are also receiving attention from private industry to set up a complete cycle for the commercialization of guayule. Guayule is bound to succeed as a commercial crop as it draws on new interest and sustained research and development.

Guayule seed quality is highly variable and has limited stand establishment by direct seeding. Seed germination is affected by dormancy of the embryo and seedcoat. Low percentages of seed containing fully developed embryos limits germination potential. Most small fields are established by transplanting. Large fields can only be economically established by direct seeding. Since the germination potential of seed is determined during seed development and production in the field, our study objective was to determine the seed quality of guayule grown under different field production systems i.e. limited irrigation, and integrated irrigation and fertilization.

SEED QUALITY EVALUATION

Introduction

Guayule (*Parthenium argentatum* Gray) is a perennial shrub that originates from the Chihuahua desert and occurs on the drylands of northcentral (Coahuila, Durango, Zacatecas, San Luis Potosi and Neuvo Leon) Mexico and in south (the Big Bend areas) and southwest (in Presidio, Brewster and Pecos counties) Texas (Lloyd, 1911; Hammond and Polhamus, 1965; West *et al.*, 1991) . It occurs naturally at an altitude of 600-2000m above sea-level and survives on sparse annual rainfall of 250-380mm. The temperature range for survival is between -18 and 49.5⁰C, and plants are tolerant of high temperature but semi-dormancy is induced by low temperature of 4⁰C (Thompson and Ray, 1989). The soils are well-drained, calcareous with a pH of 6-8 and have low fertility. Guayule produces latex that can be processed into rubber (Artschwager, 1943; Hammond and Polhamus, 1965) and the rubber is found to be non-allergenic when in contact with human tissues (Cornish and Siler, 1996). It is considered an alternative crop for production in arid and semiarid regions of the United States (Thompson and Ray, 1989; Nakayama *et al.*, 1991) and South Africa (Milthorpe *et al.*, 1991). Latex properties include strength, elasticity and viral impermeability, which makes it useful in applications where durability is required and disease transmission needs to be limited, e.g. condoms, surgical gloves, catheters (Cornish and Siler, 1996). Guayule is a vital alternative source of rubber, since increasing numbers of people show allergic responses to the current source of natural rubber, *Hevea brasiliensis* (Meuller-Agroviensis,

Euphorbiaceae) (Hamann, 1993). Processing of guayule also produces resin for adhesives, paints and varnishes (Campos-Lopez and Anderson, 1983), and antifeedant properties by termites have been shown by Gutiérrez *et al.* (1999) and Nakayama (2005).

Guayule seed is an achene, i.e. seed retained in dry ovary wall, with attached disk flowers 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 (Whitehead and Mitchell, 1943; 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 1mg each and numerous achenes are produced from the compound inflorescence (Hammond and Polhamus, 1965). The seed is enclosed in two seedcoats: (1) a soft outer coat of single cell thickness, except in the vascular bundle region, and (2) 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, so 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 to 12 months (Naqvi and Hanson, 1980). 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. Improvement of seed germination has been shown with the use of gibberellic acid in combination with planting depth and light (Hammond, 1959), and gibberellic acid and an aqueous smoke solution (Bekaardt, 2002). The active compound of the smoke solution, derived from burning plant material, was identified as furanone and was shown to promote seed germination of a wide range of species (Flematti *et al.*, 2004). These seed germination techniques were sufficient for development of nursery stock that could then be transplanted to small fields. However, when large fields are to be established the advantages of reduced labor and minimal applications in direct seeding is still more economically feasible for field establishment (Foster and Moore, 1992).

Guayule occur 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 development (Thompson and Ray, 1989).

Since the germination potential of seed is to a large extent determined during seed development and production in the field, the objective of this study was to determine the seed quality of guayule grown under different field conditions in Arizona, and with integrated irrigation and fertilization management practices.

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 was expected to be grown. Two sites in Yuma were chosen because of different soil types, with heavy clay soil in Yuma Valley and sandy loam soil in Yuma Mesa.

The line AZ-2 (PI599675GP-9) was selected for its vigorous growth and rubber yield in two years, and AZ-4 (PI599677GP-11) for its improved latex and resin content (Ray *et al.* 1999). These lines were developed in field plots at Maricopa Agricultural Center by the United States Water Conservation Laboratory USDA-ARS, Phoenix, AZ, in cooperation with the University of Arizona. The registration authority is Crop Science. 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 four lines of AZ-2, AZ-4, AZ-R2, and 11591 at four locations in Marana (32° 27' 40" N latitude, 111° 14' 00" W longitude, 601m elevation), Maricopa (33° 04' 07" N latitude, 111° 58' 18" W longitude, 361m elevation), Yuma

Mesa (32° 36' 43" N latitude, 114° 38' 02" W longitude, 58m elevation), and Yuma Valley (32° 42' 45" N latitude, 114° 42' 18" W longitude, 32m elevation) (Fig. 7). 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. Irrigation was scheduled at 80% field capacity, i.e. when soil moisture capacity reached 80%, irrigation was applied to bring soil back to full capacity. No irrigation was applied during winter when plants were dormant. A randomized complete block design with four replications was used at each location with two border rows. Each line consisted of two rows, 1m apart and 10m long, 50cm between plants and 3m between blocks. Experiment II 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. An experimental design of split block for fertility levels, split for irrigation treatments, and split for lines with six replications was used with a single border row. Each line consisted of four rows, 1m apart and 20m long, 50cm between plants and 3m between blocks. Deep neutron probes to 2m and surface TDR (time domain reflectometer) probes to 30cm were used to determine field moisture levels. Soil nitrogen levels were determined from the 0-120cm profile one month after transplanting. Average high nitrogen was at 48.81kg/ha and low nitrogen was at 16.35kg/ha. High fertility plots were established following four 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 www.ag.arizona.edu/azmet/azdata.htm.

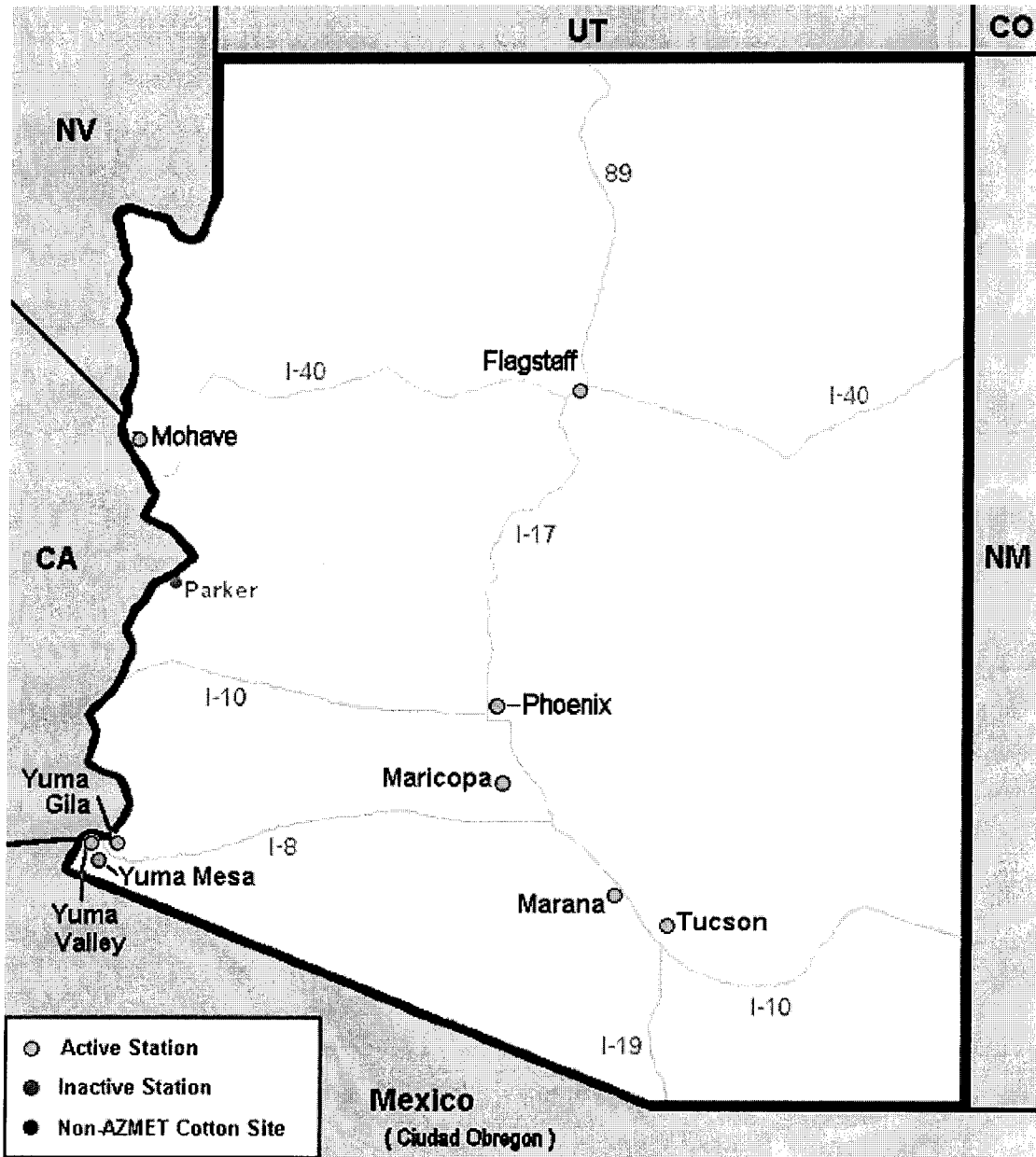


Fig. 7. Distribution of Arizona weather stations indicating trial locations at Marana, Maricopa, Yuma Mesa and Yuma Valley. (Source: www.ag.arizona.edu/azmet/azdata.htm).

Inflorescences were harvested by hand from plots during summer of 2003 and 2004. These were run through a belt thresher and blown in an air column to remove chaff. Standard germination tests (AOSA, 2002) were performed on sample units of 50 achenes per treatment per replication. Samples were placed in plastic germination boxes on water-moistened germination paper in an incubation chamber with alternating 20°C night (16 hrs) and 30°C day (8hrs) temperatures. Germination was determined after 7 days and achenes were classified as normal, abnormal, dormant, dead, and empty. Normal seedlings show the potential to develop into normal plants. Abnormal seedlings lack a radicle or cotyledons and are not expected to develop into normal plants. Empty achenes do not contain seeds. Dormant seeds do not germinate over the germination period, but remain firm and have the potential to germinate, whereas dead seeds have degraded and do not have the potential to germinate. Viability of dormant seed was determined by staining with triphenyl tetrazolium chloride. Single sections of longitudinally cut seeds of each sample were placed in a vial with 1% triphenyl tetrazolium chloride solution. The treatment was applied overnight in an oven at $\pm 35^{\circ}\text{C}$ and then evaluated as per the “Tetrazolium Testing Handbook” stipulations, i.e. even staining of cotyledons with lighter or darker staining of the embryo and radicle (Perry, 1987). These were then classified as tetrazolium positive and dead seed. Seed moisture was determined by sampling 50 seeds from each observation. The initial weight was determined by weighing the samples and then drying in an oven at 100°C. Weight remaining was recorded after 24 hours and the weight difference was calculated. Moisture content was calculated as the weight difference over initial weight and then the moisture percent was determined for each sample, i.e. on wet-weight basis.

Experiment I was analyzed as a 4x4 factorial for lines and locations, replicated in four blocks. Experiment II was analyzed as a 2x3x2 factorial for fertilizations, irrigations and lines, replicated in six blocks. The repeated measures for harvest were used as subplot factor in both experiments. Data were subjected to analyses of variance, test of normality, and regression correlation using SAS (1990).

Results

The analyses of variance for normal germination, empty achenes, and achene wet weight among lines, locations and harvests in experiment I are shown in Table 1. The normal germination, empty achenes and achene wet weight variables were identified as the main components that determine the seed quality of guayule. Significant variation in the line x location interaction occurred for normal germination, empty achenes, and achene wet weight. The highest normal germination at Marana was 43% for AZ-4 (Table 2). At Maricopa normal germination for 11591 was 54%, and at Yuma Mesa it was 56%, and at Yuma Valley AZ-4 was 59%. Empty achenes were 56% for 11591 at Marana, and 45% for AZ-2 at Maricopa (Table 3). At Yuma Mesa empty achenes were 46% for AZ-4, and at Yuma Valley AZ-2 was at 41%. Achene wet weight at Marana was 0.033g/50 achenes for AZ-R2, and 0.035g/50 achenes for 11591 at Maricopa (Table 4). At Yuma Mesa achene wet weight was 0.041g/50 achenes for 11591, and 0.041g/50 achenes for AZ-R2 at Yuma Valley. The regression correlation for normal germination vs. empty achenes was significant (Table 1), and indicates a negative relation with R^2 at 64% (Fig. 8). Correlation between normal germination and achene wet weight is positive with R^2 at 30% (Fig. 9). Data points for both correlations are distributed near the line.

Table 1. Analysis of variance for Normal Germination, Empty Achenes, and Achene Wet Weight of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

| Source | DF | Normal Germination | | Empty Achenes | | Achene Wet Weight | |
|-------------------------------------|-----|--------------------|--------|---------------|--------|-------------------|--------|
| | | MS | P | MS | P | MS | P |
| Block | 3 | 7.21 | 0.97 | 44.53 | 0.65 | 0.00003 | <0.01* |
| Location | 3 | 1077.79 | <0.01* | 1366.36 | <0.01* | 0.00037 | <0.01* |
| Line | 3 | 905.87 | <0.01* | 921.61 | <0.01* | 0.00059 | <0.01* |
| Location*Line | 9 | 545.76 | <0.01* | 542.84 | <0.01* | 0.00003 | <0.01* |
| Error (a) | 45 | 81.67 | 0.66 | 81.46 | 0.85 | 0.000008 | 0.37 |
| Harvest | 1 | 6272 | <0.01 | 5.28 | 0.83 | 0.00005 | 0.01 |
| Harvest*Location | 3 | 725.83 | 0.02 | 1111.28 | <0.01 | 0.00031 | <0.01 |
| Harvest*Line | 3 | 1331.25 | <0.01 | 262.36 | 0.08 | 0.0000002 | 0.99 |
| Harvest*Location*Line | 9 | 158.86 | 0.11 | 168.03 | 0.17 | 0.00001 | 0.11 |
| Error (b) | 48 | 92.15 | | 110.49 | | 0.000007 | |
| Corrected Total | 127 | | | | | | |
| Test for Normality | | | 0.87 | | 0.99 | | 0.09 |
| Correlation with Normal Germination | | | | | <0.01* | | <0.01* |

* imply significance at $p < 0.05$.

Table 2. Normal Germination (%) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

| LINES | LOCATIONS | | | |
|-------|------------------|-----------------|-----------------|-----------------|
| | Marana | Maricopa | Yuma Mesa | Yuma Valley |
| 11591 | 28 ^{b*} | 54 ^a | 56 ^a | 56 ^a |
| AZ-2 | 38 ^a | 34 ^b | 34 ^b | 41 ^b |
| AZ-4 | 43 ^a | 41 ^b | 35 ^a | 59 ^a |
| AZ-R2 | 42 ^a | 43 ^b | 52 ^a | 52 ^a |

* Different letters imply significance at $p < 0.05$ in comparing lines within each location.

Table 3. Empty Achenes (%) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

| LINES | LOCATIONS | | | |
|-------|------------------|------------------|-----------------|-----------------|
| | Marana | Maricopa | Yuma Mesa | Yuma Valley |
| 11591 | 56 ^{a*} | 27 ^b | 28 ^a | 22 ^b |
| AZ-2 | 46 ^b | 45 ^a | 42 ^a | 41 ^a |
| AZ-4 | 35 ^c | 36 ^{ab} | 46 ^a | 22 ^b |
| AZ-R2 | 38 ^{bc} | 34 ^b | 28 ^b | 27 ^b |

* Different letters imply significance at $p < 0.05$ in comparing lines within each location.

Table 4. Achene Wet Weight (g/50 achenes) of guayule lines AZ-2, AZ-4, AZ-R2 and 11591, from locations Marana, Maricopa, Yuma Mesa and Yuma Valley, in Arizona.

| LINES | LOCATIONS | | | |
|-------|---------------------|---------------------|---------------------|--------------------|
| | Marana | Maricopa | Yuma Mesa | Yuma Valley |
| 11591 | 0.030 ^{b*} | 0.035 ^a | 0.041 ^a | 0.040 ^a |
| AZ-2 | 0.026 ^b | 0.025 ^c | 0.029 ^c | 0.029 ^b |
| AZ-4 | 0.032 ^{ab} | 0.031 ^b | 0.033 ^b | 0.039 ^a |
| AZ-R2 | 0.033 ^a | 0.033 ^{ab} | 0.039 ^{ab} | 0.041 ^a |

* Different letters imply significance at $p < 0.05$ in comparing lines within each location.

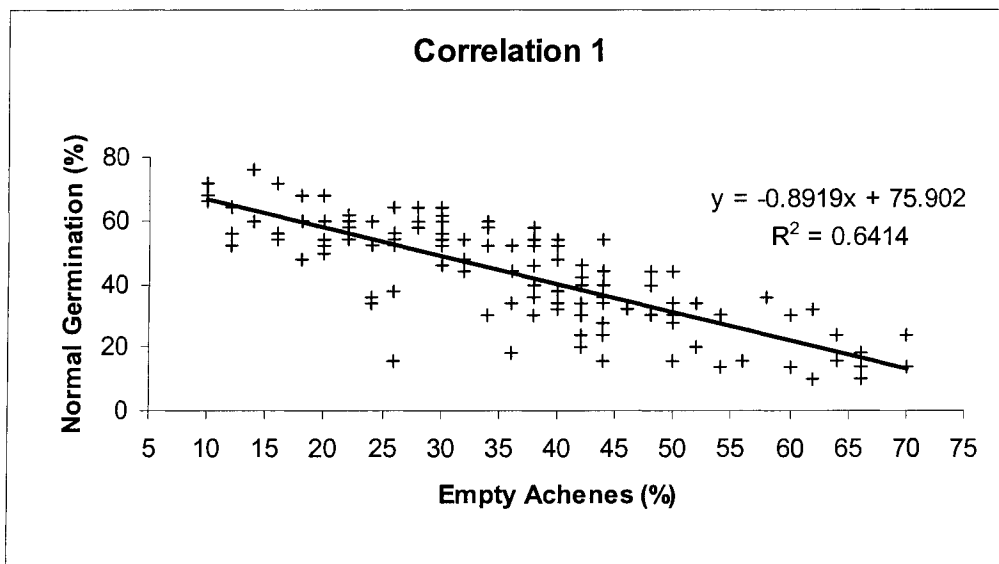


Fig. 8. Regression correlation of Normal Germination vs. Empty Achenes.

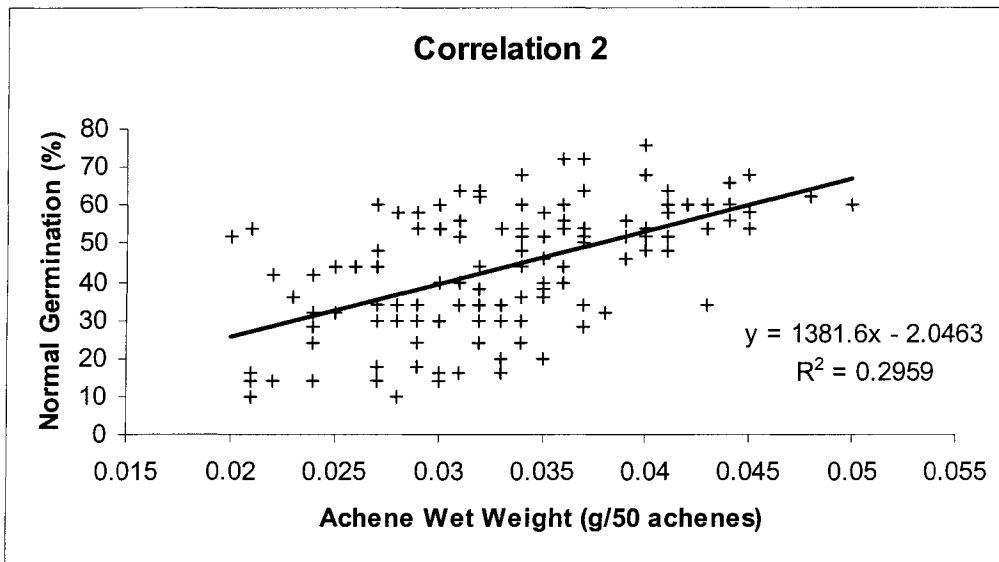


Fig. 9. Regression correlation of Normal Germination vs. Achene Wet Weight.

The analyses of variance for normal germination, empty achenes, and achene wet weight among lines, irrigations and fertilizations in experiment II are shown in Table 5. Significant variation in the line, irrigation, and fertilization occurred for normal germination. The normal germination was higher for 11591 at 66%, 55% at 60% irrigation, and 56% at high fertilization (Table 6). Significant variation in the line occurred for empty achenes. Empty achenes were at 27% for AZ-2 (Table 6). Significant variation in the two-way interactions of irrigation x fertilization, and line x fertilization occurred for achene wet weight (Table 6). Achene wet weight of the irrigation x fertilization interaction was 0.036g/50 achenes at 40% irrigation and high fertilization, and 0.032g/50 achenes at 60% irrigation and low fertilization (Table 7). The line x fertilization interaction was 0.038g/50 achenes for 11591 at high fertilization, and 0.037g/50 achenes for 11591 at low fertilization (Table 7). The regression correlation for normal germination vs. empty achenes was significant (Table 5), and indicates a negative relation with R^2 at 32% (Fig. 10). Correlation between normal germination and achene wet weight is positive with R^2 at 36% (Fig. 11). Data points for both correlations are distributed near the line.

The minimum temperature over the trial period for all locations generally followed a similar pattern (Fig. 12), with temperatures ranging between 0 and 25°C. Exceptionally low temperatures were recorded at Marana and Maricopa over the period December 2003 and February 2004. Maximum temperature was similar over the trial period for all locations (Fig. 13), with temperatures ranging from between 20 to 40°C. The average temperature (Fig. 14) rises from 20 to 30°C in March to June over the spring season. The highest temperature of 30 to 35°C occurred in summer over June, July,

Table 5. Analysis of variance for Normal Germination, Empty Achenes, and Achene Wet Weight of guayule lines AZ-2 and 11591, from location at Maricopa, in Arizona.

| Source | DF | Normal Germination | | Empty Achenes | | Achene Wet Weight | |
|-------------------------------------|-----|--------------------|--------|---------------|--------|-------------------|--------|
| | | MS | P | MS | P | MS | P |
| Block | 5 | 27.54 | 0.88 | 62.15 | 0.62 | 0.00001 | 0.16 |
| Fertilization | 1 | 3240.71 | <0.01* | 97.63 | 0.30 | 0.00052 | <0.01* |
| Irrigation | 2 | 639.27 | <0.01* | 135.40 | 0.22 | 0.00030 | <0.01* |
| Fertiliz*Irrig | 2 | 160.58 | 0.14 | 3.69 | 0.96 | 0.00004 | <0.01* |
| Line | 1 | 17408.63 | <0.01* | 5158.26 | <0.01* | 0.00299 | <0.01* |
| Fertiliz*Line | 1 | 86.47 | 0.30 | 5.06 | 0.81 | 0.00004 | 0.01* |
| Irrig*Line | 2 | 35.28 | 0.64 | 52.14 | 0.56 | 0.00001 | 0.12 |
| Fertiliz*Irrig*Line | 1 | 311.80 | 0.05 | 110.07 | 0.27 | 0.00002 | 0.06 |
| Error (a) | 48 | 77.50 | 0.86 | 87.82 | 0.22 | 0.00001 | 0.08 |
| Harvest | 1 | 21374.06 | <0.01 | 65.32 | 0.34 | 0.000001 | 0.65 |
| Harvest*Fertiliz | 1 | 3168.24 | <0.01 | 221.30 | 0.08 | 0.00004 | <0.01 |
| Harvest*Irrig | 2 | 457.66 | 0.02 | 586.25 | <0.01 | 0.00001 | 0.10 |
| Harvest*Fertiliz*Irrig | 2 | 409.23 | 0.03 | 31.40 | 0.64 | 0.000004 | 0.39 |
| Harvest*Line | 1 | 8025.56 | <0.01 | 189.34 | 0.11 | 0.00002 | 0.02 |
| Harvest*Fertiliz*Line | 1 | 17.80 | 0.68 | 12.52 | 0.68 | 0.00002 | 0.02 |
| Harvest*Irrig*Line | 2 | 58.68 | 0.59 | 262.13 | 0.03 | 0.000005 | 0.24 |
| Harvest*Fertiliz*Irrig*Line | 1 | 70.53 | 0.42 | 36.3 | 0.48 | 0.000000 | 0.89 |
| Error (b) | 51 | 106.05 | | 70.93 | | 0.000004 | |
| Corrected Total | 125 | | | | | | |
| Test for Normality | | | 0.30 | | 0.96 | | 0.64 |
| Correlation with Normal Germination | | | | | <0.01* | | <0.01* |

* imply significance at $p < 0.05$.

Table 6. Normal Germination (%) and Empty Achenes (%) of guayule lines AZ-2 and 11591, under 40%, 60% and 80% field capacity irrigation, and at high and low fertilization levels.

| VARIABLES | LINES | | IRRIGATIONS | | | FERTILIZATIONS | |
|--------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | 11591 | AZ-2 | 40% | 60% | 80% | High | Low |
| Normal Germination | 66 ^{a*} | 40 ^b | 49 ^b | 55 ^a | 50 ^b | 56 ^a | 46 ^b |
| Empty Achenes | 14 ^b | 27 ^a | 22 ^a | 20 ^a | 23 ^a | 21 ^a | 23 ^a |

* Different letters imply significance at $p < 0.05$ in comparing lines, irrigations, and fertilizations, respectively.

Table 7. Interaction of guayule lines AZ-2 and 11591, with high and low fertilization levels with respect to Achene Wet Weight (g/50 achenes).

| FERTILIZATIONS | LINES | | IRRIGATIONS | | |
|----------------|---------------------|--------------------|---------------------|--------------------|--------------------|
| | 11591 | AZ-2 | 40% | 60% | 80% |
| High | 0.038 ^{a*} | 0.029 ^b | 0.036 ^{a*} | 0.034 ^a | 0.031 ^b |
| Low | 0.037 ^a | 0.025 ^b | 0.030 ^b | 0.032 ^a | 0.025 ^c |

* Different letters imply significance at $p < 0.05$ in comparing lines within each fertilization level, and in comparing irrigations within each fertilization level.

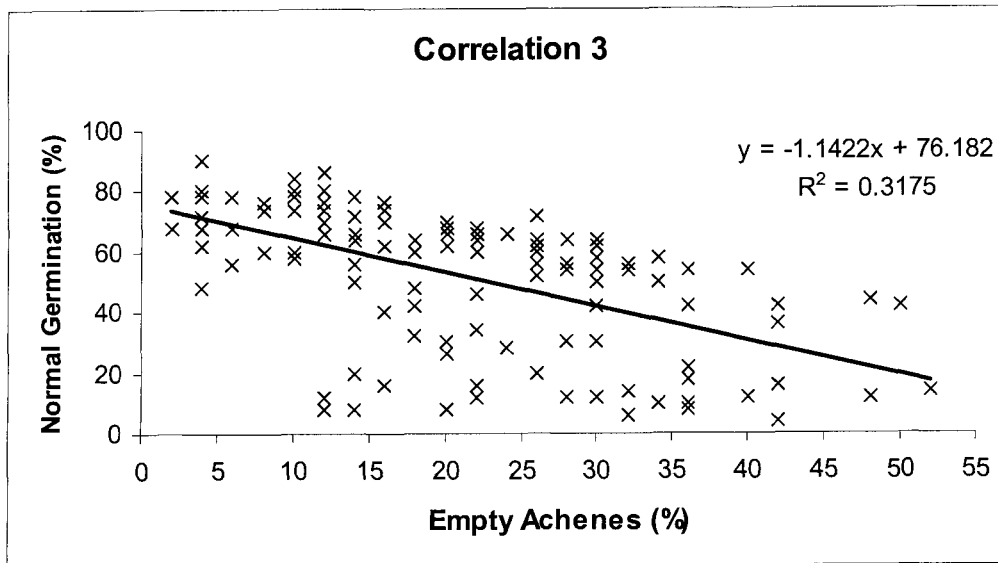


Fig. 10. Regression correlation of Normal Germination vs. Empty Achenes.

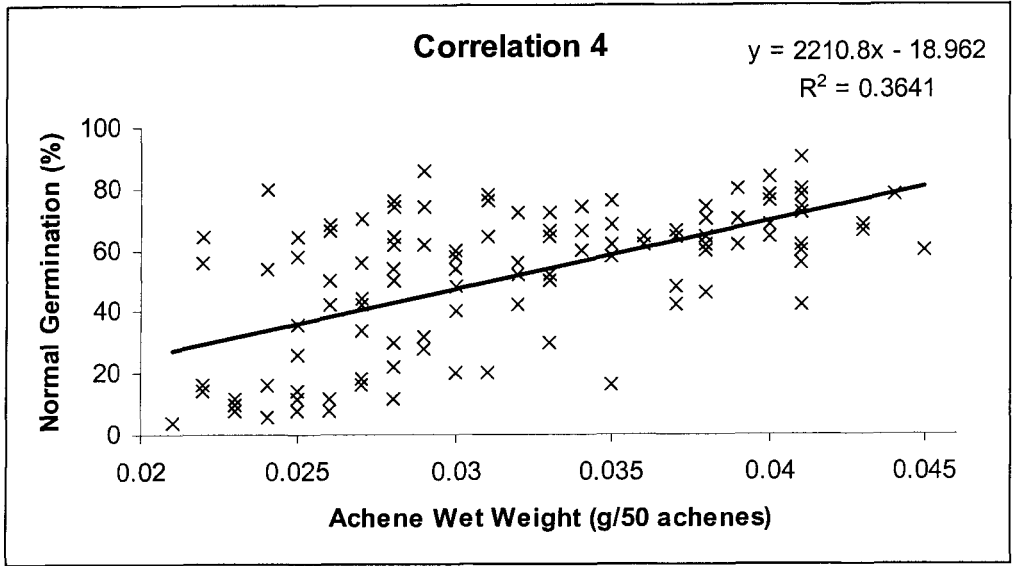


Fig. 11. Regression correlation of Normal Germination vs. Achene Wet Weight.

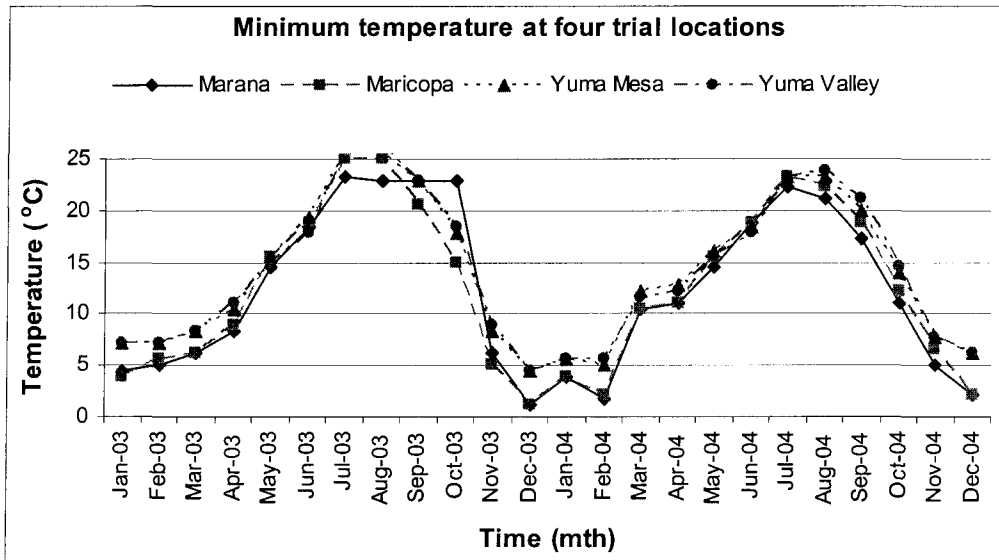


Fig. 12. Minimum monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004).

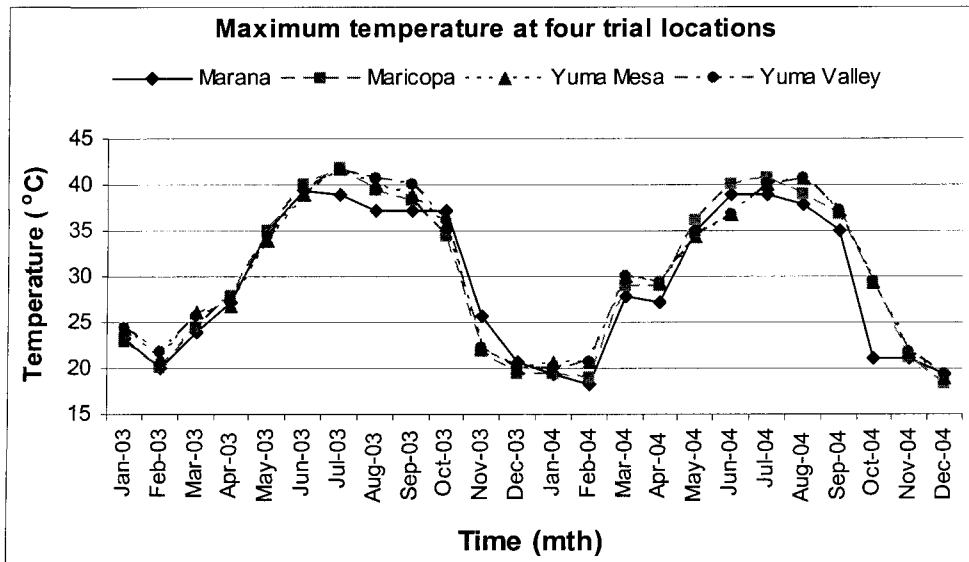


Fig. 13. Maximum monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004).

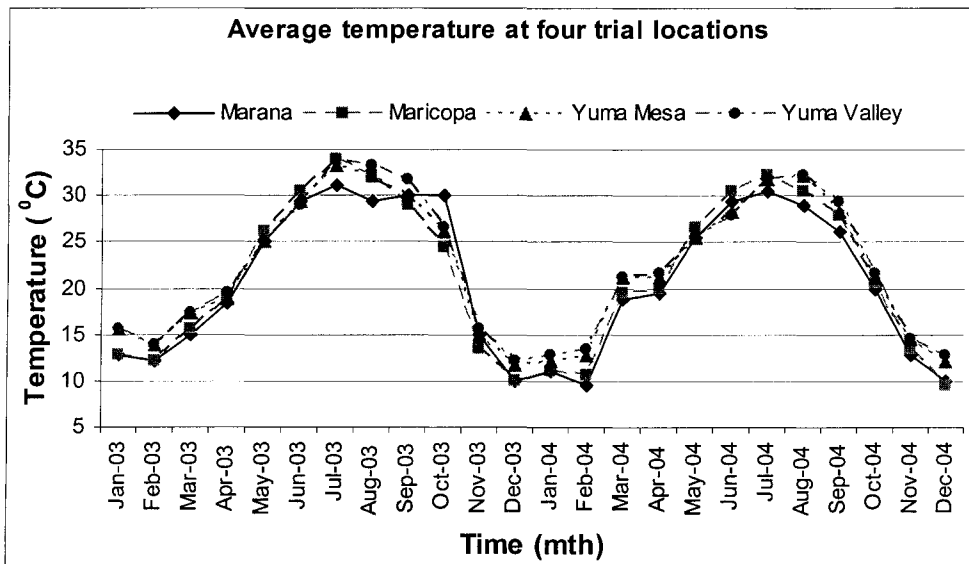


Fig. 14. Average monthly temperature at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004).

August and September. Temperature dropped from 30 to 15°C over autumn from October to December. The lowest temperature of 10°C was over winter from December to February. The maximum temperatures of 40°C would have a major effect on pollination since natural pollinators such as bees are active at 20 to 35°C (Copeland and McDonald, 1995). High temperature exposure of 30°C during flowering may limit seed-set (Foster and Moore, 1992) by increasing the rate of development to maturity, early dehiscence and resultant poor fertilization and poorly developed seed.

The total rainfall was different among locations (Fig. 15). Total average rainfall over the trial period was 167mm/pa in Marana, 145mm/pa in Maricopa, 82mm/pa in Yuma Mesa and 61mm/pa in Yuma Valley. Marana and Maricopa rainfall was higher than Yuma Mesa and Yuma Valley, with 30 to 35mm rainfall during summer in Marana and Maricopa compared to low or no rainfall in Yuma Mesa and Yuma Valley. Rainfall over the flowering period could indicate a possible limitation of seed-set as pollen is diverted from effective pollination, and bee pollinators are not active during rain or on wet flowers (Copeland and McDonald, 1995).

Wind speeds were above 8km/h at Marana, Maricopa, Yuma Mesa and Yuma Valley (Figure 16) mainly during the first bloom and less during the second bloom. Guayule reproduces under long days with continuous flowering and seed-set from late spring through summer (first bloom) and into fall (second bloom) under available moisture (Whitehead and Mitchell, 1943). Wind speeds greater than 8km/h limit effective pollination due to pollen displacement and reduction of bee pollinator activity (Copeland and McDonald, 1995). The potential limitation in pollination due to wind speed and subsequent limited seed-set thus exist at the trial locations over the trial period.

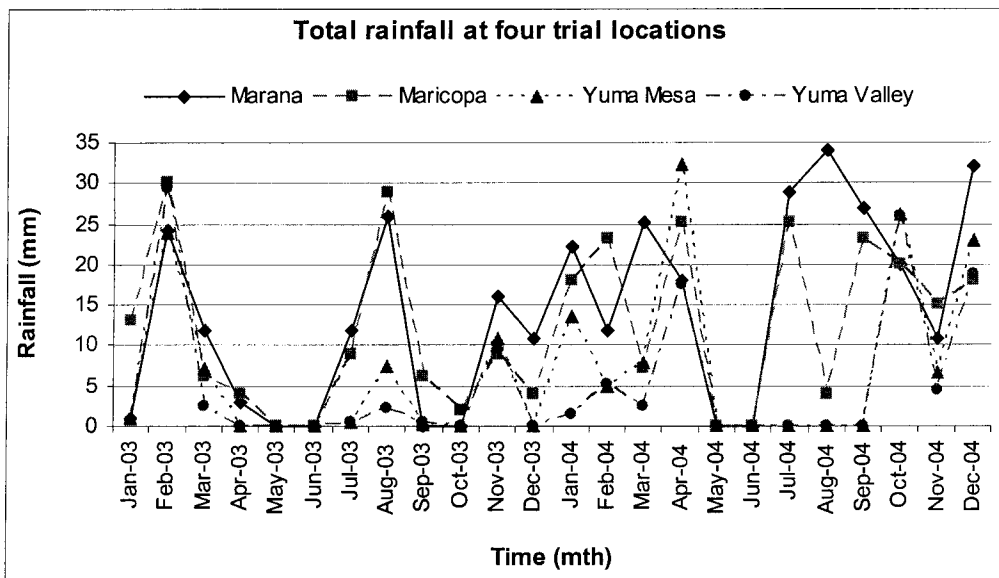


Fig. 15. Total monthly rainfall at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004).

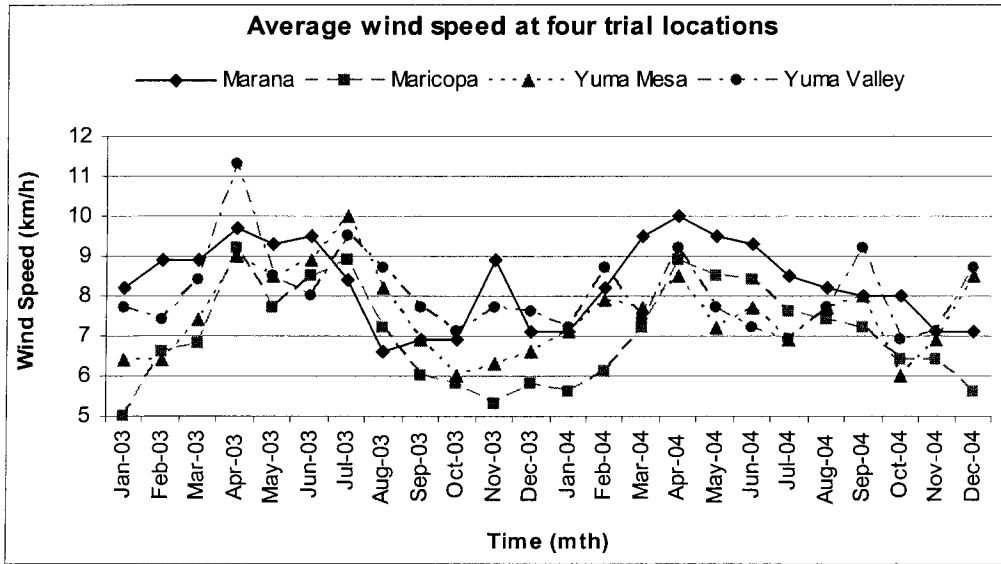


Fig. 16. Average monthly wind speed at four locations (Marana, Maricopa, Yuma Mesa, and Yuma Valley) over the trial period (January 2003 to December 2004).

Discussion

Guayule crop production in experiment I, with different locations, indicated line x location interaction. Yuma Valley was the location that produced the highest percentages of germinating seeds with line AZ-4. Seed germination for different lines was at 44% and variable among lines and locations. Empty achene production for the different lines was at 36% and variable among lines and locations. Marana had the highest empty achene production at 56%, compared to other locations. Achene wet weight for the different lines was at 0.033g/50 achenes, and variable among lines and locations. Yuma Mesa and Yuma Valley had the highest achene wet weight at 0.041g/50 achenes for 11591 and AZ-R2, respectively. Crop production in experiment II, with integrated irrigation and fertilization management, produced seed that germinated at 39%. Higher seed germination at 66% occurred with line 11591. Seed germination was 55% at 60% irrigation, and 56% at the high fertilization level. Production of empty achenes was at 31% and was higher in AZ-2. Achene wet weight was at 0.033g/50 achenes, and higher for line 11591 at high and low fertilization levels, and higher at 40% irrigation and high fertilization, and at 60% irrigation and low fertilization. The line x fertilization interaction was 0.038g/50 achenes for 11591 at high fertilization, and 0.037g/50 achenes for 11591 at low fertilization (Table 7) There is not any indication however that soil moisture and nutrient applications improves seed germination, since no significant differences, i.e. not more than 10% difference in seed germination were shown when comparing experiment I and II. Empty achene production was the major factor that decreased the germination potential of guayule seeds at all locations. Differences in seed germination and empty achene production between the different management practices

were at 5%. Comparative differences in biomass production and latex accumulation due to the larger biomass however may be obvious under different practices.

Previous studies have focused on releasing seed from dormancy and have yielded positive results with osmoconditioning, polyethylene glycol, light (Chandra, 1991), gibberellic acid and an aqueous smoke solution treatment (Bekaardt, 2002; Bekaardt *et al.*, 2004). In the newly developed guayule lines however dormancy does not greatly affect seed quality and was found to be not significant.

In our study empty achene production in both trials was the major factor that limited the germination potential of guayule seed. This was attributed to the indeterminate flowering of guayule that resulted in empty achenes being present in the harvested inflorescences. Extensive laboratory screening based on seed morphology, mass and x-ray analysis have been developed to predict the germination of seed (Jorge and Ray, 2005) and have proven successful to improve germination by selection for filled, heavier, gray and opaque-black seed. Since seed development in guayule is due to sexual and asexual, i.e. facultative apomixis reproduction, there are no shortages of means to develop seed. Also, the polyploid character of guayule could lead to complications that may arise in meiosis, i.e. chromosome reduction division, that often result in sterility. Guayule also displays sporophytic self-incompatibility and supernumerary chromosomes that impact seed formation (Thompson and Ray, 1989).

Since pollen is observed to be abundantly shed and transferred by wind and pollinators, sufficient pollen is considered to be available for pollination. However successful pollination cannot be assumed, due to excessive wind speeds and rain during flowering (Copeland and McDonald, 1995), which is an expected outcome at the trial

locations. As indicated by our experiments, empty achenes are still produced so successful pollination, fertilization and subsequent seed-set should not be assumed. Filled or viable guayule seeds have been reported to range from 10 to 45% and losses were caused by the development of embryos without fertilization, insect (*Lygus* spp.) feeding on flowers and succulent growing tips, dormancy imposed by the embryo and seedcoat, and exposure to high temperature during seed development (Foster and Moore, 1992). Since guayule is a facultative apomict, four types of seeds are produced: (1) sexual seeds, i.e. with chromosome number of reduced embryo plus that of the male gamete, (2) sexual seeds, i.e. with chromosome number of unreduced embryo plus that of the male gamete, (3) asexual seeds, and (4) polyhaploid seeds, i.e. developed parthenogenically from reduced eggs without fertilization (Estilai and Ray, 1991). The genetic variability of guayule seed production still remains the innate factor that will limit the application of direct seeding for crop establishment.

Conclusion

The seed quality of guayule produced in experiment I yielded seed germination at 44%, and in experiment II seed germination was 39%. Seed germination was affected by line x location interactions in experiment I, and the line selection, irrigation level and fertilization level in experiment II. Empty achene production in experiment I was at 36%, and 31% in experiment II. The quality of guayule seed under both trial conditions were severely decreased by empty achene production. There is not any indication that soil moisture and nutrient applications improves the seed germination potential, since no significant differences in seed germination were shown when comparing both trials. The main reason for empty achene production seems due to the genetic variability of guayule

seed production, and related to the environmental conditions of high temperature, rainfall and winds during flower bloom at the trial sites.

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