ESTABLISHMENT OF GUAYULE
(*Parthenium argentatum* Gray)

by

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DECLARATION

I state that the work done in this thesis is my own original work and that it has not been presented in part or complete at any other university for a degree.

ABSTRACT

Guayule (*Parthenium argentatum* Gray) is a semi-desert plant with the potential to become an established crop on arid land in South Africa. The plant produces latex, which can be processed into rubber that is useful in application where disease transmission needs to be limited, such as for surgical gloves and condoms. The poor germination and natural dormancy characteristics of the embryo and the seed coats of guayule seed, motivated germination experiments. Germination of seed treated with solutions of gibberellic acid, smoke water and smoke water-gibberellic acid was determined. Furthermore, combinations of gibberellic acid, smoke water and sodium hypochlorite treatment solutions were applied to seed to determine the germination responses. Vegetative propagation of guayule by means of cuttings was also investigated to determine the rooting responses of cuttings with treatment solutions of indole butyric acid, naphthalene acetamide and naphthalene acetic acid. Rooting percentage, root length and root weight was determined for each treatment. Dryland field trial plantings were established at different areas in South Africa to determine the growth potential and biomass production of guayule cultivars under different environmental conditions. Stand count, height, canopy diameter and stem diameter was determined for the different cultivars and areas. Lastly, latex production of guayule cultivars established in trial plots at Elsenburg, Oudtshoorn and Graaff-Reinet was determined after one year of growth.

Treatment solutions of an aqueous smoke extract (commonly referred to as smoke water) and gibberellic acid were evaluated to determine their effectiveness in stimulating germination of four guayule seed lines (AZ101, AZ-3, N565 and 11591). The split-plot analyses of variance showed no significant interaction between cultivar and treatment factors ($P = 0.71$), but when the day factor was included interaction was highly significant ($P < 0.0001$). The applied treatment thus had an effect on the time required for the germination response. Investigations into optimum germination responses indicated that smoke water-gibberellic acid required the shortest number of days (6.3 days) for optimum germination to occur with cultivar AZ-3. Furthermore, gibberellic acid treatment resulted in the greatest germination with the four cultivars 11591, AZ-3, AZ101 and N565, at 93.78%, 93.35%, 94.41% and 99.42% respectively. These results show

that guayule seed can be stimulated to germinate by treatment with gibberellic acid and smoke water solutions.

Specific concentrations of treatment solutions of gibberellic acid, smoke water and sodium hypochlorite, and combinations thereof were used to evaluate the germination response of guayule seed cultivar AZ-2. Combinations of treatment solutions did not result in significantly increased seed germination responses. Single treatment solutions of gibberellic acid and smoke water did not significantly enhance germination, but sodium hypochlorite however, significantly ($p < 0.0001$) suppressed germination at the 1% Cl and 2% Cl concentrations with about 5% and 10% respectively when compared to the control. Therefore, the applied seed treatments did not effectively increase the germination of guayule cultivar AZ-2 seed.

Specific concentrations of indole butyric acid, naphthalene acetamide and naphthalene acetic acid treatment solutions were applied to guayule cuttings of cultivar AZ-3 and rooting response was determined for rooting percentage, root length and root weight. Naphthalene acetic acid treatment rooted the highest percentage of cuttings (52.38%) at a concentration of 60 mg/l. Indole butyric acid treatment produced the longest roots (147.83 mm) at a concentration of 120 mg/l. Naphthalene acetamide obtained the heaviest roots (1.8 g) at a concentration of 120 mg/l. Treatment solutions of indole butyric acid, naphthalene acetamide and naphthalene acetic acid indicated specific concentrations for optimum effect to improve root formation (by 30%), root length (by 50 mm) and root weight (by 1.5 g) when compared to the controls.

Guayule trial plots of 10x10 m, rows 1 m apart and 30 cm between plants, and each cultivar (10 plants per unit) placed at random and replicated 6 times, were established in different areas under different environmental conditions in South Africa. Plantings were evaluated as a dryland practice, though irrigation was supplied only for establishment. Growth (stand count, height, canopy diameter, stem diameter) and biomass (wet and dry weight) were recorded for (1) one-year old plantings established in April 2001 at Elsenburg, Graaff-Reinet and Oudtshoorn, and (2) six-month old plantings established in October 2001 at Bethulie, Glen and Upington. Analysis of variance was done to determine mean growth and biomass for the different areas and cultivars. (1) There were significant interactions between the factors area and cultivar for stand count and height, while canopy diameter and stem diameter differences were significant only within factors. The greatest growth potential was produced by cultivars AZ-2 and AZ-3, and Oudtshoorn was the best area for growth potential and biomass production. (2) Interaction between area and cultivar was significant for plant height, but were not significant for stand count, canopy diameter and stem diameter. Cultivars produced similar results for biomass production, but were significantly different in the different areas of Bethulie, Glen and Upington. Growth potential and biomass production of guayule was influenced by the availability of water during the growth of the plant.

Latex production of guayule cultivars (AZ-2, AZ-3, N565, 11591) established in trial plots at different areas (Elsenburg, Oudtshoorn, Graaff-Reinet) in South Africa was investigated. Branch samples of one-year old plantings were harvested in April 2002, dipped in 1% ascorbate, sealed in plastic bags and chilled during airfreight to the United States Department of Agriculture (USDA) – Agricultural Research Service (ARS) in Albany CA. Latex extraction and quantification was done and mean latex production and comparisons of latex production for the cultivars in each area were determined. The evaluation of latex production show generally similar results in the different areas. Cultivars generally do not differ significantly from each other in the amount of latex produced in each area. Environmental stress factors on latex production occur in especially Oudtshoorn and Graaff-Reinet where the temperatures are above 25^{O}C and below 10^{O}C. Since guayule is a slow growing shrub, latex accumulation is also slow and takes 4-6 years to reach economic harvesting potential. Production results are therefore preliminary and require further evaluation after each year of growth to present a complete view of guayule latex production over time.

Propagation investigations were successful in identifying techniques to germinate guayule seed and promote rooting of cuttings with specialized treatment solutions. Field establishment of guayule under South African environmental conditions has identified suitable areas and indicated cultivar performances in these areas. Evaluation of the latex production of field plantings has demonstrated the potential of guayule in these areas. Currently the path to guayule development is paved with a network of research activities that is strengthened through cooperation between research institutions and private sector companies that bridge the gap between academic research and market exhibition.

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With gratitude to my family
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In loving memory of my mother
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for teaching me faith, hope and perseverance

Thank you, dear GOD
for your gifts, your guidance and your grace

CONTENT

PART 1 GUAYULE INTRODUCTION

CHAPTER I INTRODUCTION

Introduction

Guayule (pronounced why-you-lee) (*Parthenium argentatum* Gray, Compositae), is a hardy shrub native to the Chihuahua desert region (Lloyd, 1911; Hammond & Polhamus, 1965). It's economic potential lies in the latex that the plant produces, which could become a local source of natural rubber. The rubber was found to be non-allergenic when in contact with human tissues, highly elastic and strong, and blocks viral and bacterial transmission (Cornish & Siler, 1996). Guayule is a safer alternative, since a large number of people are allergic (Type I and Type IV) to the current source of natural rubber, *Hevea brasiliensis* (Meuller-Agroviensis, Euphorbiaceae). Rubber from guayule is therefore extremely useful in medical applications where durability is required and disease transmission needs to be limited, such as for the manufacture of surgical gloves, catheters and condoms (Cornish & Siler, 1996). The plant as a whole is useful in numerous applications: the plant can be used as soil stabilizer; the leaves can be used as animal feed, as compost, or wax can be extracted; the flowers can be used in fresh and dried arrangements. Resin can also be extracted from the plant for use in paints, adhesives and varnishes (Campos-Lopez & Anderson, 1983).

Guayule experimentation began in 1888 after recognizing the potential impact of a limitation in natural rubber stocks due to political disagreement, wars and increases in rubber prices. Despite extensive cultivation and intensive research under the Emergency Rubber Project there was no commercial production of cultivated guayule rubber in the world after 1962 and production from wild shrub in Mexico had also stopped. The Mexican Revolution in 1912, the sporadic wars after 1941, the Great Depression, the freezing of developmental funds and the resulting recession stopped further development (Hammond & Polhamus, 1965). New interest in guayule started in the 1970's with the rise in oil prices and resulted in the search for local resources of natural products that could alleviate the dependency on foreign markets. Investigation into guayule was funded by the National Science Foundation in 1976 and research on agronomy, breeding, rubber evaluation, economic and technology assessment was initiated by the United States Department of Agriculture in 1980 (Stewart *et al.*, 1986).

During World War II, through efforts of the United States Department of Agriculture, the first guayule seeds entered South Africa. Collaboration with Envirotech (Pty.) Ltd. and the University of Stellenbosch resulted in the establishment of guayule at Welgevallen experimental farm by Professor E.W. Laubscher in 1978. Laubscher headed a collaborative research program on guayule under the South African Council for Scientific and Industrial Research (CSIR) in 1979. This combined effort aimed to provide a local source of rubber and to provide a crop for marginal agricultural lands. Although preliminary results were encouraging, research activities on guayule declined over the years. This was partly due to the ripple effect of *Hevea* rubber remaining relatively low in price and continuously available to world markets. The latest increase in *Hevea* rubber prices and the low rand exchange value has again highlighted the need for local sources of renewable resources (Milthorpe *et al.,* 1991). Since guayule has the potential to produce similar rubber quality and strength as *Hevea* and has even better rubber properties of non-allergenicity and viral and bacterial impermeability, it warrants investigation into the potential of commercial exploitation of the plant (Cornish & Siler, 1996).

In South Africa, large areas occur with extreme temperatures where rainfall is sparse and soil nutrients are low. These areas are not suited for present commercial crops, but could be used for a crop such as guayule that thrive under extreme desert conditions. The prevailing AIDS epidemic and resulting loss of human lives also emphasizes the need for guayule products that have the ability to lessen disease transmission. These economic, environmental and social features of South Africa highlight the need for guayule research and development.

The guayule project focussed on research areas of propagation, viability and production. Techniques to encourage germination and overcome dormancy of the seed were important factors to overcome in acquiring plant material, since fresh guayule seeds exhibit natural dormancy characteristics of the embryo and seed coats (Hammond & Polhamus, 1965). Vegetative propagation of plant material by means of cuttings was also investigated to determine the potential of reproducing plant material with selected quality traits. Further investigation focused on determining the growth potential and biomass production of the different guayule cultivars under different environmental conditions in South Africa. Lastly the latex production of one-year old guayule cultivars grown at Elsenburg, Oudtshoorn and Graaff-Reinet was determined. The potential of guayule as a crop suited for marginal lands in South Africa was evaluated with regard to propagation, growth under dryland field conditions and latex production.

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CHAPTER II LITERATURE REVIEW

Introduction

Guayule (pronounced why-you-lee) (*Parthenium argentatum* Gray, Compositae), has gained renewed interest as a natural source of rubber. The main reason for this interest is due to the non-allergenic property of the rubber that is produced from the plant (Cornish & Siler, 1996). Since an increasing number of the human population, reportedly 500,000 people in the United States by 1992 (Hamann, 1993) show allergic responses to the current source of natural rubber, *Hevea brasiliensis* (Meuller-Agroviensis, Euphorbiaceae), guayule has come to the fore as a much safer alternative. The milky fluid of mixed composition (latex) found in various cell structures of the guayule plant, can be processed into rubber to manufacture vehicle tyres, shock absorbers, shoe soles, gloves, catheters, condoms, adhesives, etc (Campos-Lopez & Anderson, 1983). Guayule rubber has remarkable properties of strength, elasticity and viral impermeability, making it useful in applications where durability is required and disease transmission needs to be limited (Cornish & Siler, 1996). Also, the plant as a whole is useful in numerous applications: the plant can be used as soil stabilizer, the leaves can be used as animal feed, as compost, or wax can be extracted and the flowers can be used in fresh and dried arrangements. Resin can also be extracted from the plant for use in paints, adhesives and varnishes (Campos-Lopez & Anderson, 1983).

Initial western experimentation with guayule rubber began in 1888 after recognizing the potential impact of a limitation in natural rubber stocks due to political disagreement, wars and increases in rubber prices. After 1962 however, despite extensive cultivation and intensive research under the Emergency Rubber Project by the Intercontinental Rubber Company, there was no commercial production of cultivated guayule rubber in the world and production from wild shrub in Mexico had stopped. The main reason for the demise being the Mexican Revolution in 1912, the sporadic wars after 1941, the Great Depression, the freezing of developmental funds and the resulting recession forcing the liquidation of rubber companies (Hammond & Polhamus, 1965). The current wave of interest in guayule started in the 1970's with the rise in oil prices and resulted in the quest for local resources of natural products that could alleviate the dependency on foreign markets. Funding for investigation into guayule was provided by the National Science Foundation in 1976 and research on agronomy, breeding, rubber evaluation, economic and technology assessment was initiated by the United States Department of Agriculture in 1980 (Stewart *et al.*, 1986). In 1981 guayule also became the focus of attention in the pursuit of new crops research, development and commercialization by the Gila River Indian Community. Due to the available knowledge on the plant, greater momentum was added for guayule to be integrated into agricultural practices (Milthorpe *et al.*, 1991).

The first guayule seeds entered South Africa during World War II through efforts of the United States Department of Agriculture. Collaboration with Envirotech (Pty.) Ltd. and the University of Stellenbosch resulted in the establishment of guayule at Welgevallen experimental farm by Professor E.W. Laubscher in 1978. In 1979, Laubscher headed a collaborative research program on guayule under the South African Council for Scientific and Industrial Research (CSIR). The aim of this combined effort was to provide a local source of rubber and to provide a crop for marginal agricultural lands. Although preliminary results were encouraging, research activities on guayule declined over the years. This was partly due to the ripple effect of *Hevea* rubber remaining relatively low in price and continuously available to world markets. The latest increase in *Hevea* rubber prices and the low rand exchange value has again highlighted the need for local sources of renewable resources (Milthorpe *et al.,* 1991).

In light of the existing AIDS epidemic, in Africa specifically, there is a definite need for products such as surgical gloves, condoms, catheters and other medical equipment that come into contact with human blood and tissues. Currently used products that contain protein contaminants lead to the risk of damage to the nervous system in cases of severe allergic reactions (Type I) (Cornish & Siler, 1996). This risk of human life in itself emphasizes the need for further investigation into guayule as an alternative source of natural rubber. From an African economic perspective, it is much more cost effective to produce natural rubber and related products locally. Since there is no local source and currently natural *Hevea* rubber has to be imported, levies are incurred that must ultimately be paid for by the consumer in the purchase of the final product. Synthetic rubber on the other hand is manufactured in limited quantities that also leads to a comparatively higher purchase price (Milthorpe *et al.,* 1991), but does not have the durability and strength of natural rubber (Cornish & Siler, 1996).

The guayule research project by the Agricultural Research Council at Elsenburg started in 2000 and is a collaborative effort with the United States Department of Agriculture, under the ARS Cooperative Agreement. It aims initially to determine the techniques involved in propagating guayule from seed. Since fresh guayule seeds exhibit natural dormancy characteristics of the embryo and seed coats (Hammond & Polhamus, 1965), techniques to alleviate dormancy are important factors in acquiring plant material for the continuation of the project. Further investigation focused on the establishment and production of the plant in field conditions and understanding the agricultural practices involved in the establishment of guayule cultivars under different South African environmental conditions.

Origin

Guayule originates from the southwest part of North America. It is native to the arid to semi-arid regions of the Chihuahua desert, in particular north-central to Mexico (Coahuila, Durango, Zacatecas, San Luis Potosi and Neuvo Leon), and south (the Big Bend areas) and south-west (in Presidio, Brewster and Pecos Counties) of Texas, between $22^{O}30$' and $31^{O}30$'N longitude and 105^{O} and 100^{O}W latitude. In this ±290,000 km^2 area the plant has an irregular distribution at an altitude of 600-3000 meters above sea-level and is adept to survive on sparse annual rainfall of 250-380 mm. It occurs naturally in well-drained calcareous soils with a pH of 6-8, with daytime temperature around 35^{O}C and a minimum seldom below 0^{O}C (Lloyd, 1911; Hammond & Polhamus, 1965) (Figure 1).

Analysis of the geographical regions of South Africa indicated potential areas for guayule cultivation. The climate is generally considered mild, with no great extremes of temperature, though frost does occur in places and should be avoided as possible sites for establishment. Suitable soil structures of red sands or sandy loams on limestone underlay occur east of the Kalahari. The north-central, north-eastern and eastern regions of South Africa also have suitable medium to high fertile soils. South Africa have predominant winter- and summer rainfall patterns generally occurring from south-west to north-east of the country, with an intermediate area that benefits from both rainfall patterns. Adequate rainfall of 200-600 mm rain *per annum* occur in a band from Western Cape, across the Eastern Cape (Graaff-Reinet and Cradock), Free State and North-West (Vryburg), to the Northern Province (Smit, 1989; Milthorpe *et al.,* 1991) (Figure 2).

Classification and Morphology

CLASSIFICATION:

Parthenium argentatum (Gray) is a member of the family Compositae and the Heliantheae tribe (Lloyd, 1911).

MORPHOLOGY:

Guayule is a well branched, perennial shrub that grows to a height of about one meter (Hammond & Polhamus, 1965) (Figure 3).

Leaves: Upon germination, the first two leaves are round and dark-purple in colour (Figure 5), but quickly turn green when photosynthesis starts. Leaf ridges become lobed with age and both the dorsal and ventral surfaces are covered with T-shaped trichomes, which gives the leaves a grayish-green appearance. The leaves are at least partially deciduous, since the lower leaves shrivel up and are shed during winter drought.

Stem: The main stem is a short structure close to the ground surface. It sprouts numerous branches, which also branch further. Each of these branches ends in the formation of an inflorescence. Branches lignify (become woody due to lignin deposition) during the first year of growth.

Roots: The radicle of the germinating seed grows extremely fast to form a long taproot, from which an intricate system of dense, fibrous lateral roots and branches thereof eventually sprout. Branching roots spread out just beneath the soil surface, enabling the plant to utilize moisture of brief sporadic rains. Shallow roots that become exposed, either by erosion, digging rodents or harvesting, often form retonos (adventitious shoots) that can develop into a new plant, since it is supplied by adventitious roots that develop from the base of the retono.

Flowers: The yellow inflorescence is formed at the end of each branch as a compound, one-sided cyme (the primary axis initially bears a single terminal flower, then second order flowers develop), borne on a common receptacle. Five fertile ray-florets, each bearing a two-lobed stigma and each having two sterile disk-florets attached, line the edge of the flower head, thus forming the achene-complex. The rest of the disk-florets in the center of the head, each containing fertile stamens and an abortive pistil (stigma, style and ovary), are attached to each other at the base.

Flowering results from active growth and favourable moisture conditions, habitually during summer, but have also been observed in the nursery in young seedlings. Pollination of the flowers is caused by the wind. Insects such as bees also aid in pollination, since the outer surface of the pollen grain is sticky and spiny, and can thus adhere to a roaming insect and be transferred to a fertile stamen.

<u>Seeds:</u> Guayule seed are numerous and very small (±1mg). The achene constitutes the seed (Figure 4) that is enclosed by two seed coats:

(1) a soft outer coat of single cell thickness, except in the vascular bundle region, originating from the outer cell layer of the integument, and

(2) a tough inner coat that consist of a membrane and a one- or two-celled layer of living thick-walled endosperm cells, which is several cells thick at the micropylar end.

These two seed coats contain inhibitors (p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid) (Naqvi & Hanson, 1982) that delay germination. The mature fruit is dry, single seeded and formed from a double ovary of which only one develops into a seed (cypsela).

<u>Storage structures:</u> A milky fluid (latex) is found in all plant structures, though the greatest concentration occurs 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).

Cultivation practices

Natural reproduction to ensure the survival of guayule occurs by means of seeds and retonos.

Guayule produces a large number of small seeds per plant (Stewart & 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 seed coats (Hammond & Polhamus, 1965), which allow them to survive during periods of drought when conditions for growth is unfavourable. Seeds however have a low germination potential, as well as a high post-germination mortality (Stewart & Henderson, 1986) probably due to naturally poor seedling development properties after germination.

Retonos 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 individual 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.

Propagation from seed is presently the most cumbersome process because it involves a process of cleaning and threshing. Since harvested seed contain tightly bound disk-florets and other flower material and are tightly covered by its seed coats, it requires a mechanism of sifting that can only be achieved successfully by adjustments to a standard thresher. The thresher needs to have a close rubbing action that will break the flower clusters from each other and adequately loosen the seed coat from the seed. It also needs a screen (0.21-1.27 cm slots) that is small enough to allow only particles of specific seed size to fall through (Hammond & Polhamus, 1965). Furthermore, the embryo and the seed coats have an innate and environmental dormancy capability that keeps the seed in a resting stage. These dormancies will only be released when environmental conditions are favourable for growth or when the seed coats have been adequately degraded over time by microbial activity to allow moisture and oxygen penetration, thus activating the processes of germination. Since these activities are orchestrated by natural processes, germination is found to be spontaneous and extremely erratic. Due to the mentioned characteristics of the seed, direct seeding has been largely rejected as an effective means of propagation. Also, large quantities of seed are required for this process and considering the cost to purchase seed, it is not economically viable. Seed germination in seed trays and with special treatment solutions (Hammond, 1959) have turned out to give the most satisfactory results.

The survival rate of *seedlings* tend to be very low and could be mainly due to naturally poor seedling development properties after germination. In the nursery environment seedlings can be maintained by initially selecting a good growth medium. Selection factors that benefit seedling survival include good water filtration and aeration of the growth medium that is well above acidic pH levels (6-8). Once the seedlings have been transplanted they should be supplied with sufficient water with short frequency spray irrigation being most appropriate. Seedlings also need a source of nutrients, since the sterile growth medium of sand, peat-moss and polystyrene chips (2:1:1) does not have any nutritional value. 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. Seedlings are prone to aphid and snail feeding, but these can be easily controlled by selective spraying and snail bait respectively.

Vegetative propagation by means of cuttings primarily involves taking ±5 cm terminal cuttings, treating it with a rooting hormone (such as indole butyric acid, naphthalene acetic acid, naphthalene oxyacetic acid or naphthalene acetamide - in liquid or powder form) (Hammond & Polhamus, 1965) and planting it in a growth medium. 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 & 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; Radin, 1984). Benzyladenine (1 mg/L) was found to stimulate shoot growth, while indole butyric acid (0.5 mg/L) initiate 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 has been identified.

Guayule planting is mainly determined by the availability of water. Since it is envisioned to manage guayule as a dryland crop, consideration is given to the rainfall pattern in the area. In Australia's winter-rainfall areas planting is generally scheduled for late August to mid September (spring planting), when there is greater chance of rain during September, October and November. Planting in early March to mid April (autumn planting) is more appropriate in summer-rainfall areas to make use of soil moisture from fallen rain during December to February (Stewart & Henderson, 1986). Irrigation should be applied weekly after initial planting up to establishment for about one month, until the plant is successfully rooted and is able to draw water from the soil. Plants are commonly placed in rows of 1-1.2 meters apart and with 30-50 centimeters between plants. Row spacing is mainly determined by available machinery for weed control and harvesting, while spacing between plants is governed by aspects such as the selected cultivar's growth form (more upright than spreading) and whether seed will be collected manually or mechanically.

Agricultural development

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, coarse to medium textured soils in the pH region of 6-8 (Hammond & Polhamus, 1965). Transplanted seedlings are prone to initial drowning and require an obstruction free area for optimal root penetration during initial establishment, which could be attained by deep ploughing (150 - 200 mm). Any land investigated for guayule establishment should thus be selected with consideration of these characteristics.

Fertilization is not considered a crucial crop management tool since guayule has a low nutrient requirement and is thus specifically implemented in areas with poor soil types. It follows naturally however that an increase in available sources of nutrient would increase biomass production. Fifty kilograms of nitrogen and 20kg phosphorous per hectare is given as a guide on Australian soils, noting that the plant will use what nutrients there are available in the soil. Field experiments have yet to prove that related costs of soil fertilization can be justified in relation to the final raw product, which is accumulated latex (Ferraris, 1986).

Weed infestation in newly planted guayule fields directly relates to the spectrum of past weed occurrence and seed deposits. This is extremely important during the establishment of the young plant, due to its slow growth rate. A number of commercial herbicides such as oxyfluorofen (Goal), oryzaline (Surflan) and DCPA (Dacthal) have been tested in trial plantings with positive results (Whitworth, 1981; Milthorpe, 1984). Since no broad-spectrum weed control agent has yet been declared safe for use in guayule, the best approach to weed control is governed by good agricultural practices. Simple weed control can be done by mowing between rows and hand-weeding between plants, thus ensuring that the crop is not overgrown by or is adversely affected through competition with the weeds, while also preventing soil exposure to erosion.

Diseases in the soil are mostly due to poor water filtration and can therefore be remedied by selecting soil with good drainage for guayule planting. Soil-borne diseases usually cause root rot that terminates the plant's ability to draw water and nutrients from the soil. Related diseases include verticillium wilt caused by the soil fungus *Verticillium albo-arum,* which attacks the roots and interfere with water supply and induce wilting of tops, phymatotrichum root rot that is caused by *Phymatotrichum omnivorum,* which affects the roots, and cause sudden wilting, drying and curling of the leaves, and the fungus *Sclerotium batatacola* causing charcoal or crown rot that appears as black and dark brown sunken lesions and cause dying of the tops. Seedling root rot is caused by *Phythium ultimum,* which attacks the taproot or root crowns and causes pinkish or reddish colour of woody cylinders at lesions (Hammond & Polhamus, 1965).

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 & Polhamus, 1965). The ripe guayule seeds are borne in the flowerheads and loosen from the cluster when disturbed. A number of mechanical seed harvesters have been used by the Intercontinental Rubber Company that functions on a vacuum system for seed collection (Hammond & Polhamus, 1965). Improvements on this basic design incorporated a pressure and suction blower for seed collection also from the ground (Stewart & Henderson, 1986), to prevent the accumulation of other debris. Other designs include a harvester with a vibrator to dislodge seed and a blower to move seed to suction inlets (Tysdal, 1981). Coates (1985) also 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).

Rubber harvesting of guayule involves two approaches: (1) complete removal of the plant (branches and roots) after 5-6 years of growth, as employed in the Emergency Rubber Project and (2) ratoon cropping (clipping of the branches 50-100 mm above ground level) after two years of growth (Lloyd, 1911), and then complete harvesting of the re-grown shrub after 6-7 years (Hunter *et al.*, 1959). Guayule shrub harvesting equipment first used by the Intercontinental Rubber Company focussed on complete removal of the plant by digging and used a two-row digger mounted behind a tractor (Hammond & Polhamus, 1965). Further development of equipment incorporated the idea of clipping (pollarding), with different cutting tools having been evaluated by Coates (1986) and investigation on the regrowth in response to clipping (Garrot & Ray, 1983). Harvesting time takes into consideration the growth stage of the plant, having predominant stages of active growth and a dormant period. 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). It is thus suggested that plants be harvested during the dormancy stage (habitually in winter) after active growth has stopped. This timing diminishes 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 also be given to the chilling-requirement of the plant in its production of latex (Bonner, 1943; Benedict, 1950). Though it has not yet 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.

Latex production

Guayule is a highly versatile shrub due to the products that become available from processing. Though rubber is the main product and latex a refined derivative thereof, resin can also be obtained during the extraction process. Wax can also be extracted from leaves and the bagasse can be used as burning fuel (Gartside, 1986). Latex is found in most plant parts, i.e. leaves, branches and roots, and is contained in individual cells. The greatest amount of latex is found in the bark, in vascular rays of the phloem and xylem. Lesser amounts are formed in pith, xylem parenchyma and epithelial cells of the resin canals. Very small amounts are present in the leaf parenchyma and in the peduncles (Artschwager, 1943). Guayule is habitually adept to survive in hot and dry semi-desert areas. High light intensity, as is provided by the prevailing sunshine, was found favourable for the production of latex on soils with high nutrient levels, though plants grown in low nutrient soils were unaffected (Mitchell *et al.*, 1944). Interestingly though, the plant's ability to tolerate the extreme night cold in these environments is also significant in latex production (Van Staden *et al.*, 1986). Latex has been shown to be produced under warm day ($\pm 27^{O}C$) and low night ($\pm 7^{O}C$) temperatures (Bonner, 1943). The availability of soil moisture also plays a role in latex production. During seasonal availability of water (or irrigated conditions), active summer growth results in greater biomass production (vegetative growth). This increased production of xylem tissue during active growth however does not enhance latex production in phloem tissue of mature plants (Lloyd, 1911; Artschwager, 1943). Water stress conditions usually result in latex accumulation, presumably as a survival mechanism.

A number of *extraction procedures* have been developed over the years in the processing of guayule. The initial chewing of the guayule shrub by native Americans and developments through the Emergency Rubber Project (Hammond & Polhamus, 1965) has advanced into a finely tuned procedure focussed on maximum product quantity (latex from bagasse) and quality (rubber from resin) extraction. The scarcity of water in arid regions where guayule plantations are envisioned, the cost related to the construction of a processing facility, the infrastructure required for storage of the harvested crop and the preservation of rubber quantity and quality in plants during storage (Taylor & Chubb, 1952) are formidable challenges that face the successful commercialization of guayule. The most commonly employed methods of extraction are based on the following three basic concepts:

1. Flotation involves boiling the shrub in water and passing it through a mill to chop the shrub into smaller pieces. The chopped shrub is then treated with a caustic solvent that ruptures the cell walls, allowing latex to be released in strands. These strands are then skimmed from the slurry and treated with acetone to remove resin from the rubber. This process produces rubber that contains no natural antioxidants and thus has no protection against oxidation. Rubber is dissolved in hexane to allow the removal of debris by filtration and the addition of antioxidants, such as P-p-diamino-diphenyl-methane and phenyl-beta-napthyl-amine, before drying and storing to prevent degradation. The hexane is then removed by steam ventilation, leaving behind only the extracted rubber product.

2. Sequential extraction was specifically developed to improve the quality of the rubber by improving the separation of resin from rubber (Hamerstrand & Montgomery, 1984). A polar solvent such as acetone is used for deresination of the ground shrub, which can also be recycled and used as the extraction medium. Rubber is then removed from the resin-free slurry by extraction with a solvent such as hexane. Antioxidants can be added to the solvent from the moment of extraction. Rubber extraction can be done by immersion, gravity percolation or by counter-current percolation procedures to obtain the final rubber product.

3. *Simultaneous extraction* relies heavily on the efficiency of the solvent to extract both rubber and resin, and to release the rubber upon addition of a polar "rubber non-solvent" (Wagner & Parma, 1988). Halogenated solvents such as methyl chloride, perchloroethylene and 1,1,1-trichloroethane can serve as rubber/resin solvents, while methanol and ethanol can be used as the non-solvent (Wagner & Schloman, 1991). Further research developments by Weihe and Nivert (1980) for the Firestone Tire and Rubber Company produced a suitable model for simultaneous shrub deresination and solvent extraction of rubber (Figure 6).

Conclusion

Guayule, as a benign plant of the Chihuahuan desert, has experienced years of extensive - though continuously revisited - investigations since 1888. Of all other inquired domestic sources of natural rubber, guayule was found useful in not just producing rubber of comparable quality and strength to *Hevea*, but also distinguished itself in producing rubber with non-allergenic properties and viral impermeability, which makes it extremely valuable in the prevention of disease transmission. Added to this, the whole plant is useful in producing resin, cork, wax, and bagasse.

As a crop suited for dryland production, guayule has the potential to be incorporated into agricultural practice in areas that can not be utilized for existing crop production due to a lack of sufficient rainfall and poor soil. Since the plant is able to survive extreme temperatures (hot and cold) and does not require intensive soil fertilization, there is great potential to establish farming activities on marginal lands. Technology is currently available on plant physiology, genetics, breeding, propagation, cultivation and agronomy, harvesting and processing to successfully manage guayule as a crop. At the other end of the spectrum, the development of products and markets are also receiving attention from private industry to set up a complete cycle for the commercialization of guayule.

The potential of guayule commercialization in South Africa is vast, especially considering the large areas of suitable land available for production, as well as the economic implications of producing natural rubber locally. Though research expertise on the agricultural practice of guayule is available through collaborative efforts, it needs to be explored and amended for South African environmental conditions and crop management strategies. Research and development of new guayule lines to increase production is a continuous effort to ensure the feasibility of guayule as an alternative crop. From an economic perspective guayule cultivation requires minimal input and low maintenance, but also requires the construction of facilities capable of handling the extraction and processing of products. As with any emerging venture, guayule requires initial drive from private sector, development by industry and a sound commitment by government to be actively involved in the revival of South African agriculture.

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Figures

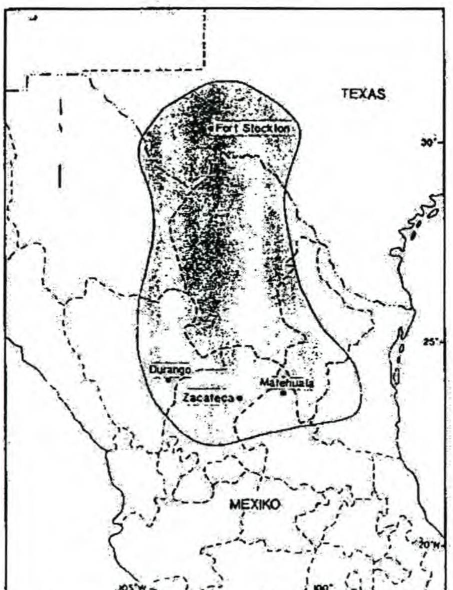

Figure 1. Geographic distribution of native Guayule (Héctor-Gómez-Contreras 1978)

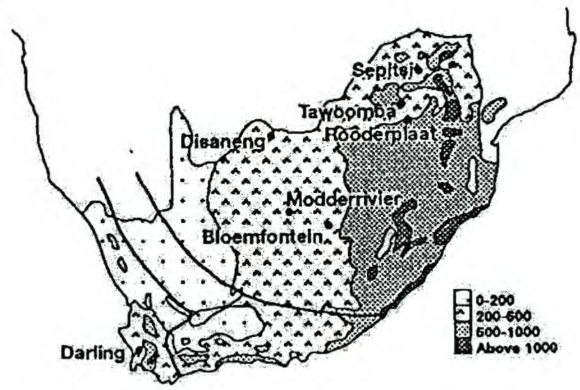

Figure 2. Distribution of rainfall and potential areas for Guayule establishment in South Africa (Whitworth and Whitehead 1991)

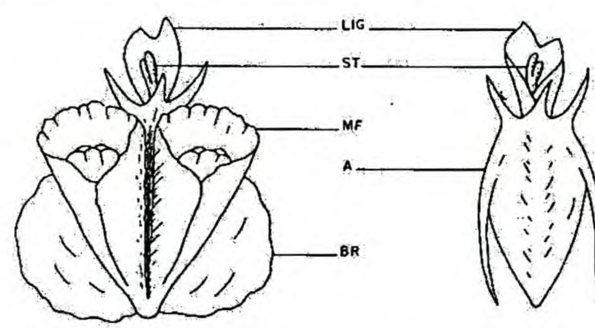

Figure 4. Diagram of achene-complex of Guayule
LIG – ligule, ST – 2-lobed stigma, MF – male (disk) florets, A – achene, BR – subtending bract
(Hammond and Polhamus 1965)

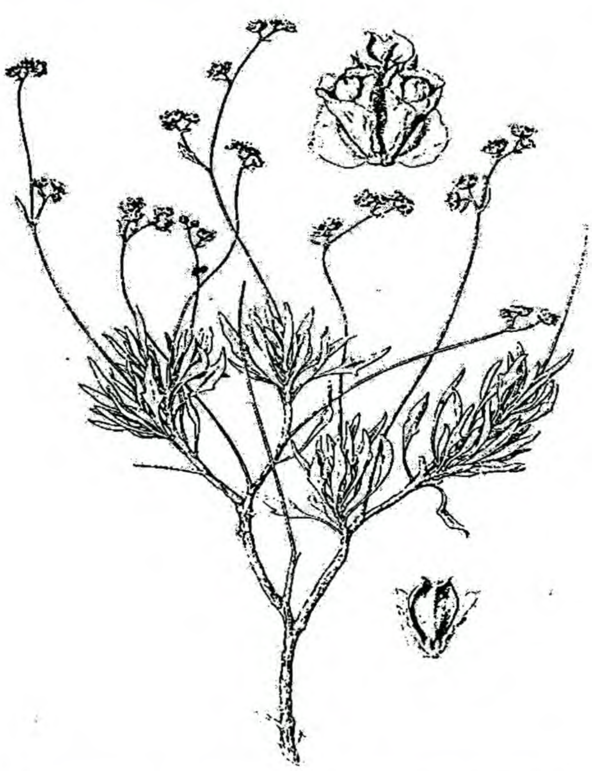

Figure 3. Diagram of Guayule branching, leaves, inflorescence and seed (Hammond and Polhamus 1965)

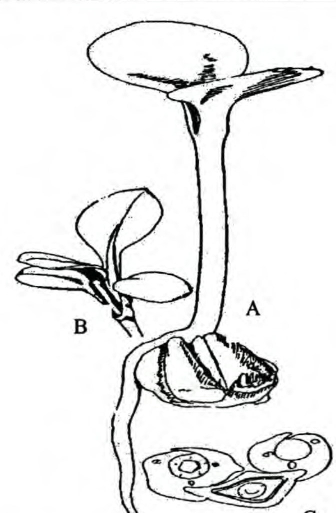

Figure 5. Diagram of Guayule seedling
A – fully germinated seedling; B – cotyledons and first two foliage leaves; C – transverse section through achene of a ray flower and two attached disk flowers (Lloyd 1911)

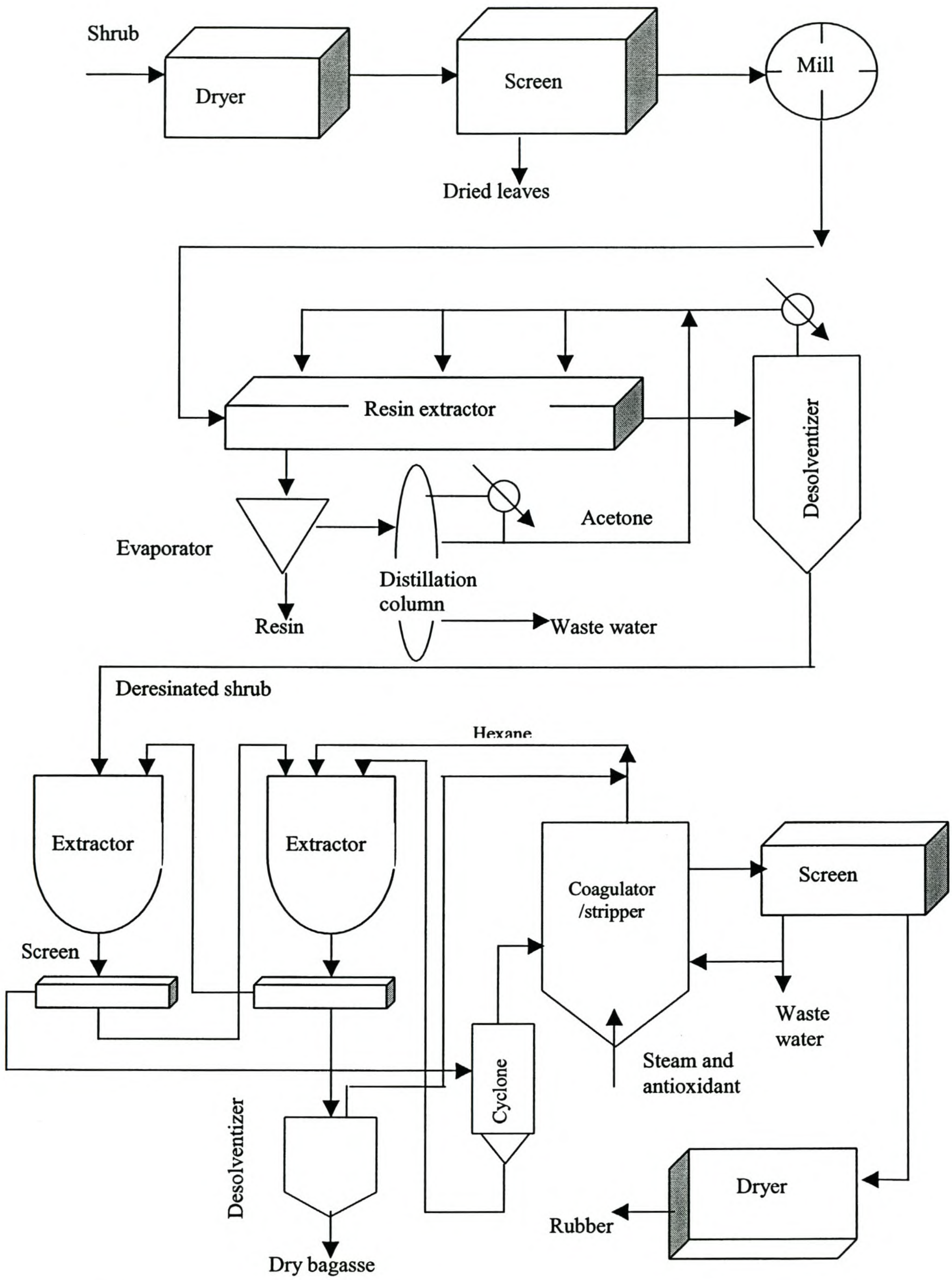

Figure 6. Flow diagram of shrub deresination followed by rubber extraction (Stewart and Lucas 1986)

PART 2 GUAYULE PROPAGATION

CHAPTER III INVESTIGATION OF TREATMENT SOLUTIONS ON GUAYULE SEED GERMINATION

Abstract

Guayule seed exhibit poor germination due to natural dormancy of the embryo and the seed coats that result in a state of rest that can last from six- to twelve months after seed-set. In order to overcome this drawback, techniques to encourage seed germination were investigated. Treatment solutions of an aqueous smoke extract (commonly referred to as smoke water) and gibberellic acid were evaluated to determine their effectiveness in stimulating germination of four guayule seed lines (AZ101, AZ-3, N565 and 11591). The split-plot analyses of variance showed no significant interaction between cultivar and treatment factors ($P = 0.71$), but when the day factor was included interaction was highly significant ($P < 0.0001$). The applied treatment thus had an effect on the time required for the germination response. Investigations into optimum germination responses indicated that smoke water-gibberellic acid required the shortest number of days (6.3 days) for optimum germination to occur with cultivar AZ-3. Furthermore, gibberellic acid treatment resulted in the greatest germination with the four cultivars 11591, AZ-3, AZ101 and N565, at 93.78%, 93.35%, 94.41% and 99.42% respectively. These results show that guayule seed can be stimulated to germinate by treatment with gibberellic acid and smoke water solutions.

Keywords: gibberellic acid, guayule, *Parthenium argentatum*, seed germination, smoke water

Introduction

Parthenium argentatum Gray, Compositae, commonly known as guayule (pronounced why-you-lee), belongs to the Compositae family and originates from the drylands of Texas and Mexico (Hammond & Polhamus, 1965). Its commercial importance is due to the source of natural rubber that can be produced from the latex in the plant. Latex occurs in individual cells throughout the plant and is accumulated in the primary cortex, parenchymous cells, pith cells and vascular rays of young plants, but in the vascular rays of the xylem and phloem of mature plants (Artschwager, 1943). The produced rubber was found to have non-allergenic properties (Cornish & Siler, 1996), which is of extreme importance considering that many people are allergic (Type

IV and Type I) to the current source of natural rubber, *Hevea brasiliensis* (Meuller-Agroviensis, Euphorbiaceae). Guayule products thus have a specialized application in the medical field for the manufacturing of surgical gloves, catheters and condoms in the prevention of disease transmission. Other applications include the extraction of resin for use in paints, adhesives and varnishes (Campos-Lopez & Anderson, 1983).

Guayule forms yellow inflorescence at the end of each branch as a compound, one-sided cyme borne on a common receptacle. Five fertile ray-florets line the edge of the flower head and each bear a two-lobed stigma and have two sterile disk-florets attached. This forms the achene-complex. The rest of the disk-florets in the center of the head are attached to each other at the base and each contain fertile stamens and an abortive pistil (stigma, style and ovary) (Hammond & Polhamus, 1965). Active growth results in flowering during favourable moisture conditions habitually during summer. The wind and insects such as bees cause pollination of the flowers, since the pollen grain is light and the outer surface is sticky and spiny. Guayule seed are numerous and very small (±1mg each). The achene constitutes the seed and is enclosed in two seed coats: (1) a soft outer coat of single cell thickness, except in the vascular bundle region, originating ~~from the outer~~ cell layer of the integument, and (2) a tough inner coat that consist of a membrane and a one- or two-celled layer of living thick-walled endosperm cells, which is several cells thick at the micropylar end. These two seed coats contain inhibitors (p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid) that delay germination (Naqvi & Hanson, 1980). The mature fruit is dry, single seeded and formed from a double ovary of which only one develops into a seed (cypsela). Seed propagation is presently a cumbersome process involving a process of seed cleaning and threshing. Since harvested seed contain tightly bound disk-florets and other flower material and are tightly covered by its seed coats, it requires a mechanism of sifting that can only be achieved successfully by adjustments to a standard thresher. The thresher needs to have a close rubbing action that will break the flower clusters and adequately loosen the seed coat from the seed. It also needs a screen (0.21-1.27 cm slots) that is small enough to allow only particles of specific seed size to fall through (Hammond & Polhamus, 1965). Furthermore, the embryo and the seed coats have an innate and environmental dormancy capability that keeps the seed in a resting stage. These dormancies will only be released when the seed coats have been adequately degraded over time by microbial activity to allow moisture and oxygen penetration and when environmental conditions are favourable for growth. This activates the processes of germination development of the embryo. Activation of catabolic enzymes (gibberellic acid in the aleuron layer), make nutrients (in endosperm) available to the embryo for germination (Kigel & Galili, 1995). Since these activities are orchestrated by natural processes germination is found to be initially very much delayed and eventually spontaneous and extremely erratic. Due to the mentioned characteristics of the seed, direct seeding has been largely rejected as an effective means of propagation. Also, large quantities of seed are required for this process and considering the cost to purchase seed, it is not economically feasible (Erickson & Smith, 1947).

Since guayule seeds exhibit natural dormancy characteristics of the embryo and seed coats (Hammond & Polhamus, 1965), treatment solutions were investigated to determine seed germination responses. Seed germination in seed trays and with special treatment solutions (Hammond, 1959) have yielded the most satisfactory results. Brown (1993) initially tested an aqueous smoke extract on fynbos species and reported enhanced seed germination. Brown *et al.* (1993) found that a plant-derived smoke extract promoted seed germination of Cape Erica species, and Brown *et al.* (1994) reported similarly for Restionaceae. In this experiment we endeavour to evaluate the ability of smoke water and gibberellic acid to stimulate seeds of guayule to germinate. We thus record the general effectiveness of the treatments and the responsiveness of the cultivars for germination to occur. Also, we record the peak number of days and peak germination percentages resulting from interaction with the different treatments and cultivars.

Materials and methods

Ten grams of each seed cultivar (AZ101, AZ-3, N565 and 11591), obtained from United States Water Conservation Laboratory (USWCL), was soaked in 100 ml treatment solution and placed in a growth chamber (UNIEQ) under fluorescent lighting (3x Philips TL40W/33RS) at ±22°C for 12 hours. Seeds were then planted in peat cups filled with sterile growth medium [river sand: peat moss: polystyrene chips (2:1:1)] and placed on heated beds (±25°C) with a sprinkle irrigation schedule of 1min each hour from 7:00 to 19:00.

The applied treatments were 1. Gibberellic acid (GA) – 100mg gibberellic acid (GA3) per liter distilled water, 2. Smoke water (SW) – aqueous smoke extract marketed as Fire Grow™, 3. Smoke water-Gibberellic acid (SW-GA) – 500 ml smoke water solution + 500 ml gibberellic acid solution, 4. Water (CON) – distilled water and 5. None (NONE) – no treatment. A split-plot analysis of variance on percentage germination of seed was used. The main plot was a 4x5 factorial with factors 4 cultivars and 5 treatments randomly replicated in 4 blocks. The sub-plot was time (5, 10, 15 and 20 days from treatment). An experimental unit consisted of 36 seeds. The germination incidence was transformed to percentages and logits before subjecting to analysis of variance. Second order polynomial regressions were fitted on the percentage germination over time and the optimum number of days (time required for most seed to germinate) and optimum percentage germination (greatest germination responses) were determined and subjected to factorial analyses of variance (SAS, 1999).

Results and discussion

Table 1 Analysis of variance of guayule seed (AZ101, AZ-3, N565, 11591) germination responses with treatment solutions of gibberellic acid (GA), smoke water (SW) and smoke water-gibberellic acid (SW-GA)

The GLM Procedure					
Dependent Variable: PGERM					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
Block	3	3602.889	1200.9629	2.74	0.0519
CULT	3	5747.372	1915.791	4.36	0.0078
TREAT	4	99863.38	24965.84	56.87	<.0001
CULT*TREAT	12	3898.775	324.898	0.74	0.7068
DAY	3	89057.36	29685.788	163.71	<.0001
DAY*CULT	9	10828.92	1203.213	6.64	<.0001
DAY*TREAT	12	24551.94	2045.99	11.28	<.0001
DAY*CULT*TREAT	36	17959.06	498.86	2.75	<.0001

The split-plot analyses of variance showed no significant interaction between cultivar and treatment factors ($P = 0.71$), but when the day factor was included interaction was highly significant ($P < 0.0001$) (Table 1). The applied treatment thus had an effect on the time required for the germination response.

Investigations into the optimum number of day response (Figure 1) show that seed treatment with smoke water-gibberellic acid solution resulted in the shortest time required for germination. Cultivar AZ-3 had the shortest time of 6.3 days followed by cultivar 11591 at 6.86 days, N565 at

7.74 days, and with cultivar AZ101 taking exceptionally longer at 9.74 days. Treatment with gibberellic acid solution showed optimum day response in 9.0 days for cultivars 11591 and N5653, though cultivar AZ-3 and AZ101 took longer at 12.7 and 11.4 days respectively. Treatment with smoke water solution, control and no treatment resulted in comparatively longer optimum day responses, with distinctions of cultivar responses, especially 11591 responding in 7.9 days. The four cultivars thus had significantly different responses to the different treatment solutions to reach optimum day germination.

Investigations into the optimum germination response (Figure 2) show that gibberellic acid solution resulted in the greatest germination for the four cultivars 11591, AZ-3, AZ101 and N565, at 93.78%, 93.35%, 94.41% and 99.42% respectively. Gibberellic acid is an enzyme present in the aleuron layer of a seed and is primarily responsible for the breakdown of endosperm, thus causing germination (Kigel & Galili, 1995). The increased supply of gibberellic acid in the seed treatment solution thus enhanced seed germination. Hammond (1959) had also reported on the enhancement of guayule seed germination with gibberellic acid treatment. Smoke water-gibberellic acid treatment resulted in the second best germination response with cultivar AZ-3 at 84.00%, followed by smoke water treatment with cultivars AZ-3 at 81.53%, N565 at 81.17% and 11591 at 80.60% germination.

Treatment with smoke-water-gibberellic acid solution also resulted in optimum germination of cultivars AZ101 at 76.74%, 11591 at 75.86% and N565 at 75.36%. Earlier tests done to evaluate the effectiveness of a plant-derived smoke extract on the germination of seed also concluded positive results (Brown *et al.*, 1993; Brown *et al.*, 1994). Baxter and Van Staden (1994) also reported on the effectiveness of using a smoke extract as pre-treatment on *Themeda triandra* (Forssk, Poaceae) seed to overcome seed dormancy. Control and no treatment solution resulted in lower optimum germinations compared to the test solutions. Results obtained from this experiment show that guayule seed is stimulated to germinate by treatment with gibberellic acid and smoke water solutions. Cultivars differ in their responses to the different seed treatments, in the optimum number of days and optimum germination responses.

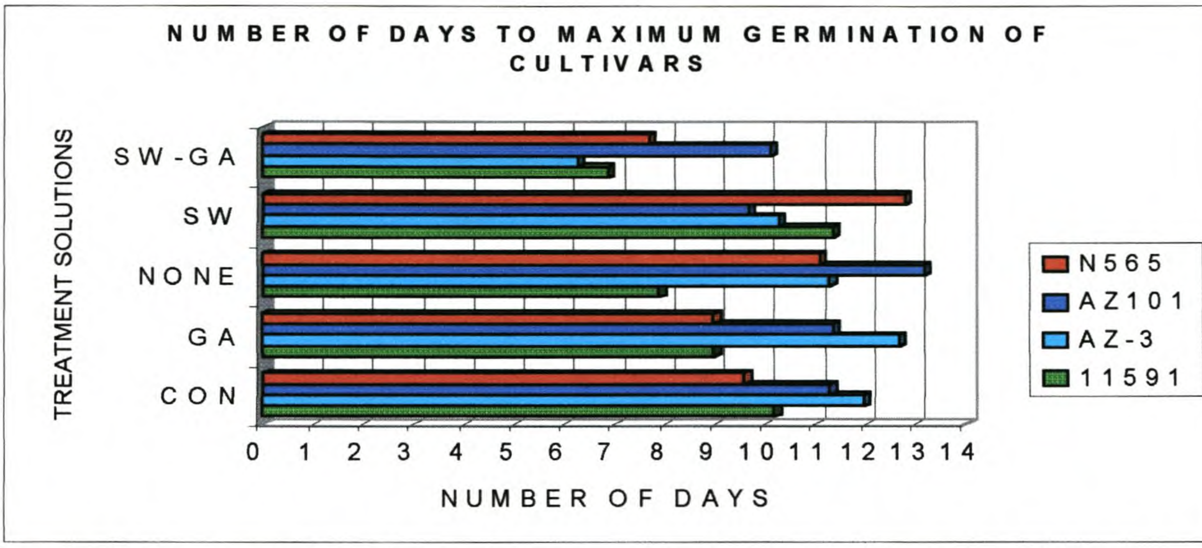

Figure 1 Optimum number of days for seed germination of guayule cultivars in response to applied treatments of gibberellic acid (GA), smoke water (SW), smoke water-gibberellic acid (SW-GA), no treatment (NONE) and the control (CON). Least significant difference (lsd) for treatment = 2.6389 and lsd for cultivar = 2.3603.

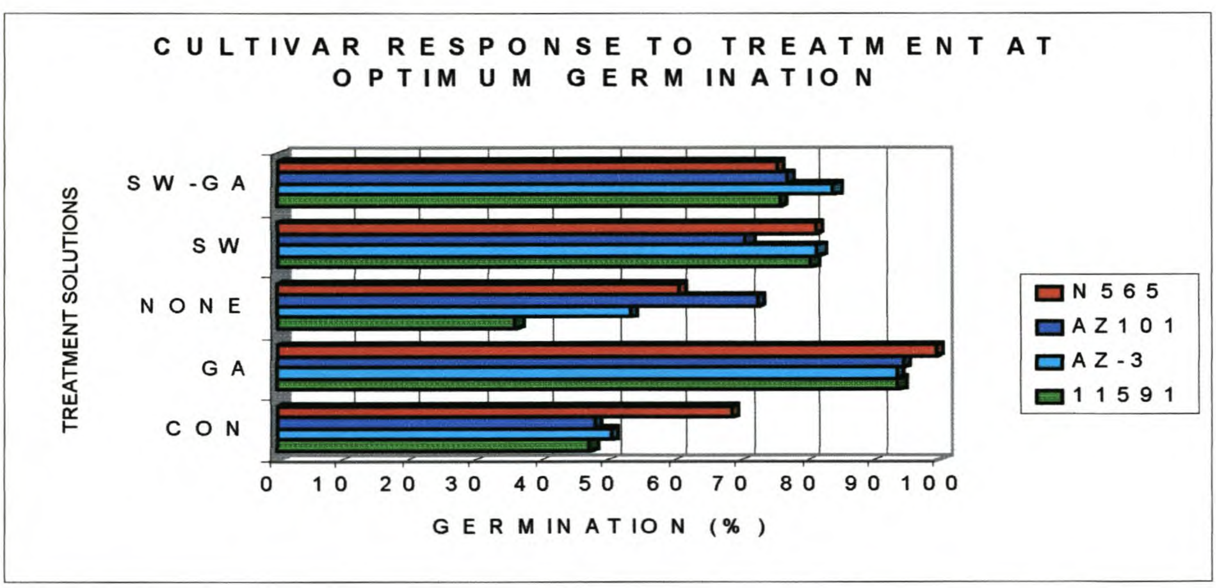

Figure 2 Optimum seed germination of guayule cultivars in response to applied treatments of gibberellic acid (GA), smoke water (SW), smoke water-gibberellic acid (SW-GA), no treatment (NONE) and the control (CON). Least significant difference (lsd) for treatment = 9.2121 and lsd for cultivar = 8.2581.

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We would like to thank the United States Department of Agriculture for funding of the project and special thanks to Terry Coffelt at the US Water Conservation Laboratory for providing the guayule seed.

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CHAPTER IV GUAYULE SEED GERMINATION RESPONSES WITH SPECIFIC CONCENTRATIONS AND COMBINATIONS OF TREATMENT SOLUTIONS

Abstract

The poor germination of guayule (*Parthenium argentatum* Gray) seed is a factor that limits propagation of the shrub. Specific concentrations of treatment solutions of gibberellic acid, smoke water and sodium hypochlorite, and combinations thereof were used to evaluate the germination response of guayule seed cultivar AZ-2. Combinations of treatment solutions did not result in significantly increased seed germination responses. Single treatment solutions of gibberellic acid and smoke water did not significantly enhance germination, but sodium hypochlorite however, significantly ($p < 0.0001$) suppressed germination at the 1% Cl and 2% Cl concentrations with about 5% and 10% respectively when compared to the control. Therefore, the applied seed treatments did not effectively increase the germination of guayule cultivar AZ-2 seed.

Keywords: gibberellic acid, guayule, *Parthenium argentatum*, seed germination, smoke water, sodium hypochlorite.

Introduction

Guayule (*Parthenium argentatum* Gray, Compositae) is a benign shrub that originates from the Chihuahua desert on the drylands of southwest Texas and northcentral Mexico (Lloyd, 1911; Hammond & Polhamus, 1965). Extensive research and development have been done on guayule since the discovery that the plant produces latex that can be processed into rubber (Artschwager, 1943; Hammond & Polhamus, 1965). Furthermore, the rubber was found to be non-allergenic (Cornish & Siler, 1996) when in contact with human tissues, thus accentuating its market potential in the medical field. The plant also produces resin that can be used in adhesives, paints and varnishes (Campos-Lopez & Anderson, 1983).

A yellow inflorescence is formed at the end of each branch as a compound, one-sided cyme, borne on a common receptacle. Fertile ray-florets bear two-lobed stigmas and sterile disk-florets that form the achene-complex. Disk-florets in the center of the head each contain fertile stamens and an abortive pistil (stigma, style and ovary) and are attached to each other at the base (Hammond & Polhamus, 1965). Flowers are formed due to active growth during favourable moisture conditions habitually during summer. Pollination is caused by the wind and insects, since the pollen grain is light and the outer surface is sticky and spiny, and can thus be blown away and adhere to a roaming insect and be transferred to a fertile stamen. A large number of very small (±1 mg each) guayule seed are produced. The achene constitutes the seed that is enclosed by two seed coats: (1) a soft outer coat of single cell thickness, except in the vascular bundle region, originating from the outer cell layer of the integument, and (2) a tough inner coat that consist of a membrane and a one- or two-celled layer of living thick-walled endosperm cells, which is several cells thick at the micropylar end. These two seed coats contain inhibitors (p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid) that delay germination (Naqvi & Hanson, 1980). The mature fruit is dry, single seeded and formed from a double ovary of which only one develops into a seed (cypsela). Seed propagation involves a process of cleaning and threshing, since harvested seed contain tightly bound disk-florets and are tightly covered by its seed coats. Cleaning and threshing of seed requires a mechanism of sifting that can only be effective by adjustments to a standard thresher. The thresher needs to have a close rubbing action that will break the flower clusters from each other and adequately loosen the seed coat from the seed. The screen (0.21-1.27 cm slots) needs to be small enough to allow only particles of specific seed size to fall through (Hammond & Polhamus, 1965). Furthermore, the embryo has an innate and environmental dormancy capability that keeps the seed in a resting stage and will only begin germinative development when the seed coats have been adequately degraded over time by microbial activity and environmental conditions are favourable for growth. The penetration of moisture and oxygen through the seed coats activate enzymatic activity that releases the seed from dormancy. Germination in wild populations is found to occur after six to twelve months and is extremely erratic, since germination activation occur by natural processes. Direct seeding has been largely rejected as an effective means of propagation due to the mentioned characteristics of the seed (Erickson & Smith, 1947). Also, large quantities of seed are required for direct seeding and considering the cost to purchase seed, it is not economically viable. Seed germination in seed trays and with special treatment solutions (Hammond, 1959) have yielded the most promising results.

Guayule development has always been limited by poor seed propagation. Fresh guayule seeds enter a stage of dormancy that is apparent in the embryo (2 months) and seed coats (6 to 12 months) (Hammond & Polhamus, 1965). Efforts to enhance seed germination have identified gibberellic acid, sodium hypochlorite (Hammond, 1959; Naqvi & Hanson, 1980) and smoke water (Brown, 1993; Baxter & Van Staden, 1994) as effective seed treatment solutions. This experiment investigates the germination response of guayule cultivar AZ-2 seed to specific concentrations and combinations of gibberellic acid, smoke water and sodium hypochlorite treatment solutions.

Materials and method

Clean seed of guayule cultivar AZ-2, obtained from United States Water Conservation Laboratory (USWCL), were soaked in 15 ml of each treatment solution for 2 hours and then rinsed with distilled water. Each treatment, consisting of one hundred seeds, was kept on moist filter paper in a petri dish and incubated in a growth chamber (UNIEQ) under fluorescent lighting (3x Philips TL40W/33RS) at ±22°C. The applied treatments were gibberellic acid (GA) (2000 ppm, 1000 ppm, 500 ppm, 0 ppm), smoke water (SW) – an aqueous smoke extract marketed as Fire Grow™ (200 ml/l, 100 ml/l, 50 ml/l, 0 ml/l) and sodium hypochlorite (NaOCl) (2% Cl, 1% Cl, 0.5% Cl, 0% Cl) and each treatment was replicated twice. Combinations of the treatment solutions were prepared as per Table 1, generating 64 treatment units and resulting in a 4x4x4 factorial experiment. The number of seed germinated (radicle emergence) was determined after 15 days by visual inspection. Percentages of seed germination were calculated and analysis of variance was determined (SAS, 1999).

Table 1 Treatment solution combinations of gibberellic acid (GA), smoke water (SW) and sodium hypochlorite (NaOCl) for testing of germination response of guayule seed

Number	GA	SW	NaOCl
1	0	0	0
2	0	0	0.5
3	0	0	1
4	0	0	2
5	0	50	0
6	0	50	0.5
7	0	50	1
8	0	50	2
9	0	100	0
10	0	100	0.5
11	0	100	1
12	0	100	2
13	0	200	0
14	0	200	0.5
15	0	200	1
16	0	200	2
17	500	0	0
18	500	0	0.5
19	500	0	1
20	500	0	2
21	500	50	0
22	500	50	0.5
23	500	50	1
24	500	50	2
25	500	100	0
26	500	100	0.5
27	500	100	1
28	500	100	2
29	500	200	0
30	500	200	0.5
31	500	200	1
32	500	200	2
33	1000	0	0
34	1000	0	0.5
35	1000	0	1
36	1000	0	2
37	1000	50	0
38	1000	50	0.5
39	1000	50	1
40	1000	50	2
41	1000	100	0
42	1000	100	0.5
43	1000	100	1
44	1000	100	2
45	1000	200	0
46	1000	200	0.5
47	1000	200	1
48	1000	200	2
49	2000	0	0
50	2000	0	0.5
51	2000	0	1
52	2000	0	2
53	2000	50	0
54	2000	50	0.5
55	2000	50	1
56	2000	50	2
57	2000	100	0
58	2000	100	0.5
59	2000	100	1
60	2000	100	2
61	2000	200	0
62	2000	200	0.5
63	2000	200	1
64	2000	200	2

Results

Table 2 Analysis of variance of guayule seed germination with treatment solution combinations of gibberellic acid (GA), smoke water (SW) and sodium hypochlorite (NaOCl)

The GLM Procedure					
Dependent Variable: PGERM					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
Block	1	50.000	50.000	0.880	0.353
GA	3	192.563	64.188	1.120	0.346
SW	3	106.750	35.583	0.620	0.603
GA*SW	9	411.813	45.757	0.800	0.616
NaOCl	3	2109.813	703.271	12.320	<.0001
GA*NaOCl	9	469.750	52.194	0.910	0.519
SW*NaOCl	9	206.063	22.896	0.400	0.930
GA*SW*NaOCl	27	1443.125	53.449	0.940	0.562

The two and three way interactions of gibberellic acid, smoke water and sodium hypochlorite treatment solutions were not significant for seed germination (Table 2). Different concentrations of sodium hypochlorite influenced germination significantly ($p < 0.0001$) (Figure 1). Treatment with smoke water ($p = 0.603$) and gibberellic acid ($p = 0.346$) did not have significant effect on seed germination (Figure 2 and 3).

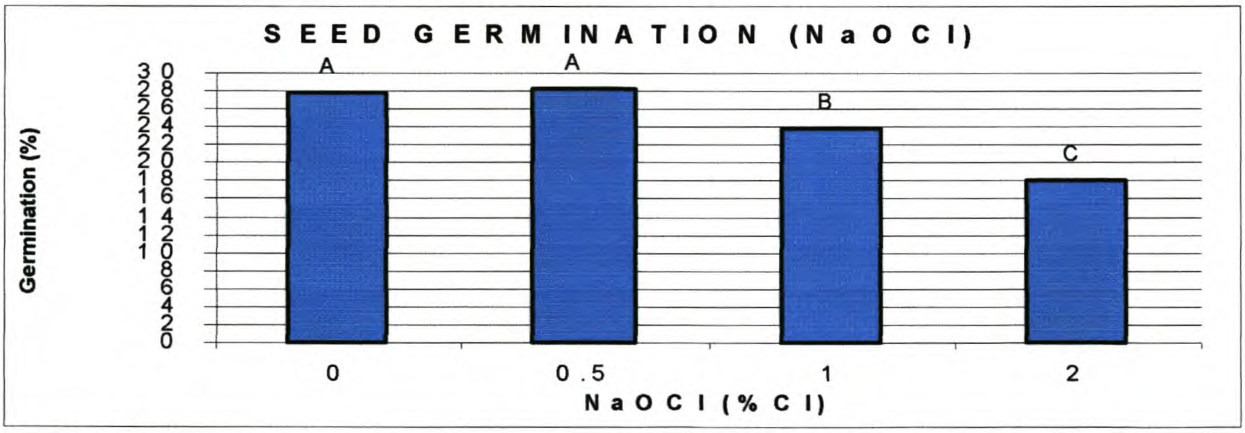

Figure 1 Germination responses of guayule seed to specific concentrations of sodium hypochlorite (NaOCl). Least significant difference (lsd) = 3.7744. Different letters indicate significant differences ($p = 0.05$).

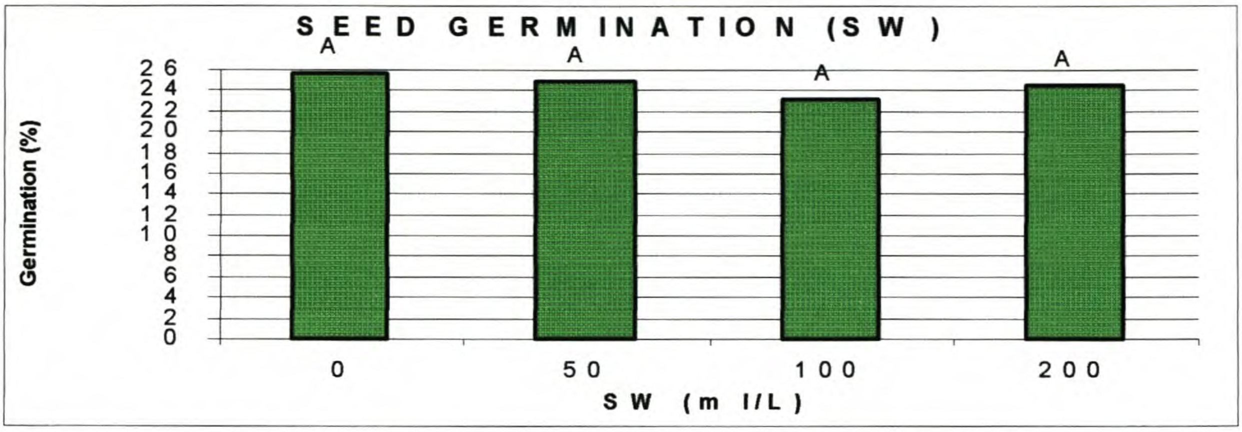

Figure 2 Germination responses of guayule seed to specific concentrations of smoke water (SW). Least significant difference (lsd) = 3.7744. Different letters indicate significant differences (p = 0.05).

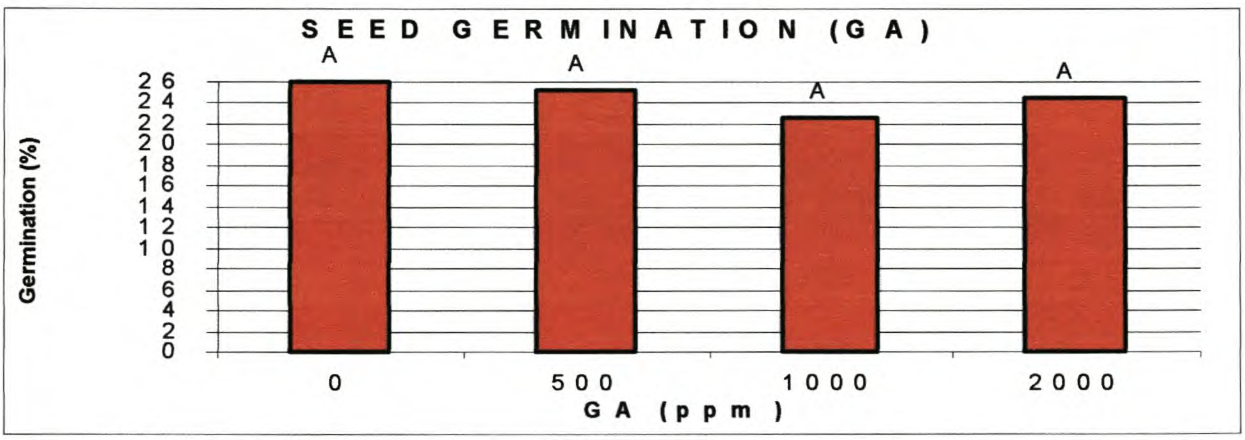

Figure 3 Germination responses of guayule seed to specific concentrations of gibberellic acid (GA). Least significant difference (lsd) = 3.7744. Different letters indicate significant differences (p = 0.05).

Discussion

The combination effects of gibberellic acid, smoke water and sodium hypochlorite treatment solutions did not yield significant interactions for seed germination (Table 2). The only significant differences ($p < 0.0001$) was found with the sodium hypochlorite treatment solutions. Guayule seed of cultivar AZ-2 had a negative germination response to treatment with increased concentrations of sodium hypochlorite (Figure 1). Germination was suppressed to 23.88% at the 1% Cl concentration and to 18.16% at the 2% Cl concentration. At the 0% Cl concentration (control) 28.31% seed germination resulted, indicating a significant decrease in germination of

4.44% and 10.16% respectively. Treatment with different concentrations of smoke water (p = 0.603) and gibberellic acid (p = 0.346) did not result in any significant improvement of seed germination in comparison to the controls (25.59% and 25.94% respectively). Figure 2 and 3 show a slight decrease in seed germination with increased concentrations.

Reports on the effectiveness of smoke water to encourage seed germination had been presented by Baxter and Van Staden (1994), and Brown (1993) reported on the promotion of seed germination for fynbos species. Naqvi and Hanson (1980) also noted the improvement of guayule seed germination with combinations of high concentrations of gibberellic acid and low concentrations of sodium hypochlorite. The improvement of guayule seed germination with smoke water, gibberellic acid, and a combination of smoke water and gibberellic acid treatments was also shown in my earlier investigation on seed germination with cultivars AZ-3, AZ101, N565 and 11591. Significant improvement of guayule seed germination with gibberellic acid, smoke water and sodium hypochlorite could not be proven in this experiment for cultivar AZ-2.

The large number of small (±1 mg each) seed produced by guayule inevitably present high variability in seed quality. Freshly harvested guayule seed quickly enters a state of dormancy that can last from six to twelve months (Hammond & Polhamus, 1965). Therefore the age of seed at harvesting and the storage conditions of seed after harvesting affect the viability of seeds. Since no details are available about the duration and conditions of seed storage, strong dormancy characters or poor seed quality could result in poor germination. The levels of endogenous inhibitors (p-hydroxybenzoic acid, protocatechuic acid, p-coumaric acid and ferulic acid) in guayule seed (Naqvi & Hanson, 1980) also influence the germination response. Furthermore, the protective double seed coat layer and dormancy character of the embryo (Hammond & Polhamus, 1965) naturally equips the seed for long periods of survival in a state of rest. The innate embryo and external environmental factors influence germination not only as single units, but also in conjunction with each other (Kigel & Galili,1995). All these factors are imposing considerations in the task to improve the germination potential of new guayule seed cultivars. The variability of guayule seed quality needs to be further investigated to produce consistent germination responses with proven treatment solutions, especially to evaluate new seed cultivars.

Acknowledgements

We would like to thank the United States Department of Agriculture for funding of the project and special thanks to Terry Coffelt at the US Water Conservation Laboratory for providing the guayule seed.

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CHAPTER V ROOTING RESPONSES OF GUAYULE CUTTINGS TO TREATMENT SOLUTIONS

Abstract

The vegetative propagation of guayule (*Parthenium argentatum* Gray) has merit for multiplication of selected mother-plants with elite qualities of vigorous growth and high latex yield. This experiment investigates the rooting responses of guayule cuttings to treatment solutions of indole butyric acid, naphthalene acetamide and naphthalene acetic acid. Specific concentrations of indole butyric acid, naphthalene acetamide and naphthalene acetic acid treatment solutions were applied to guayule cuttings of cultivar AZ-3 and rooting response was determined for rooting percentage, root length and root weight. Naphthalene acetic acid treatment rooted the highest percentage of cuttings (52.38%) at a concentration of 60 mg/l. Indole butyric acid treatment produced the longest roots (147.83 mm) at a concentration of 120 mg/l. Naphthalene acetamide obtained the heaviest roots (1.8 g) at a concentration of 120 mg/l. Treatment solutions of indole butyric acid, naphthalene acetamide and naphthalene acetic acid indicated specific concentrations for optimum effect to improve root formation (by 30%), root length (by 50 mm) and root weight (by 1.5 g) when compared to the controls.

Keywords: guayule, indole butyric acid, naphthalene acetic acid, *Parthenium argentatum,* vegetative propagation

Introduction

Research and development of guayule (*Parthenium argentatum* Gray, Compositae), a desert plant native to the Chihuahua desert (Lloyd, 1911; Hammond & Polhamus, 1965), is focussed on improving the suitability of the plant for commercial utilization as a natural source of latex. Since the discovery that the plant produces latex that can be processed into rubber (Artschwager, 1943; Hammond & Polhamus, 1965), many other properties of the plant have been discovered. The plant also produces resin that can be used in adhesives and varnishes (Campos-Lopez & Anderson, 1983) and the rubber is non-allergenic to human tissues (Cornish & Siler, 1996). Breeding programs have identified desirable characteristics to select for crop improvement, i.e. increase of latex yield and quality, improved ability to regrow, environmental stress resilience, resistance to pests and diseases (Estalini & Ray, 1991). The quest to reproduce desired traits emphasizes the need for techniques to multiply selected mother-plant material vegetatively by means of cuttings. Natural vegetative propagation occurs under field conditions when branches and roots become separated from the mother-plant. 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 to produce an independent plant (Lloyd, 1911). Vegetative propagation procedures basically involve taking cuttings from the mother-plant and treating it with a hormone such as indole butyric acid to stimulate root formation (Hammond & Polhamus, 1965). Though vegetative propagation is considered impractical for large-scale cultivation practices (Erickson & Smith, 1947), there is a need to be able to produce plants with similar characteristics be it only as a starting point for basic research. In this experiment we investigate the rooting response of guayule cultivar AZ-3 cuttings to specific concentrations of indole butyric acid, naphthalene acetamide and naphthalene acetic acid treatment solutions.

Materials and method

Terminal cuttings of 12 cm length and ±3 mm stem thickness of guayule cultivar AZ-3 were obtained during summer from 2.5-year old plantings at Elsenburg in the Western Cape (±34^{0}S and 19^{0}E). About 200 mother-plants were sampled and a maximum of ten cuttings was taken from a single plant. These were dipped in diathane (2 g/l) for two minutes to discourage fungal growth and left to air-dry for ten minutes. Leaves were stripped from the middle to the base of the stem and about 1cm of the base was cut away to obtain a fresh wound for absorption of the treatment solution over twelve hours.

The applied treatment solutions were indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA). Dilutions of 120 mg/l, 60 mg/l, 30 mg/l, 15 mg/l and 0 mg/l were prepared for each treatment. Cuttings were then planted in speedling trays filled with sterile growth medium [river sand: peat moss: polystyrene chips (2:1:1)] and placed on heated beds (±25°C) with a sprinkle irrigation schedule of 1 min each hour from 7:00 to 19:00.

Fifteen treatment units were generated with each unit consisting of 21 cuttings placed at random and replicated in four blocks. The experiment was a 3x5 factorial design and root formation, root length and root weight were determined after 8 weeks. Analysis of variance was determined for the rooting response to the different treatments (SAS, 1999).

Results

The treatment solution and concentration showed significant ($p = <0.001$) interaction for rooting percentage of guayule cuttings (Table 1). Indole butyric acid treatments tended to decrease root formation with an increase in concentration and at 15 mg/l concentration resulted in the highest percentage rooting (Figure 1). Naphthalene acetamide treatments resulted in the highest rooting at 30 mg/l and 120 mg/l concentrations. A similar trend was observed with naphthalene acetic acid treatments with concentrations of 15 mg/l and 60 mg/l resulting in the best rooting responses. Naphthalene acetic acid treatment rooted the highest percentage of cuttings at a concentration of 60 mg/l, in comparison to indole butyric acid and naphthalene acetamide (least significant difference (lsd) = 17.751). Treatment solutions at optimum concentrations improved rooting by 20% to 30% when compared to the control, which resulted in 15% to 19% rooting.

The treatment solution and concentration showed a significant ($p = 0.0107$) interaction for root length of guayule cuttings (Table 2). Naphthalene acetamide treatments produced the longest roots at 30 mg/l and 120 mg/l concentrations (Figure 2). The indole butyric acid treatments had no effect on root length with increase in concentration, with the exception at 120 mg/l concentration that produced the longest roots. Naphthalene acetic acid had very similar results to indole butyric acid, i.e. increased root lengths at 30 mg/l, 60 mg/l and 120 mg/l concentrations. Indole butyric acid treatment produced the longest roots (147.83 mm, at a concentration of 120 mg/l) in comparison to naphthalene acetamide and naphthalene acetic acid (lsd = 29.283). The control resulted in 50 mm to 80 mm mean root lengths, which was improved with 30 mm to 50 mm at optimum treatment solution concentrations.

Table 1 Analysis of variance for rooting (%) of guayule cuttings with treatment solutions indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	10606.198	623.894	4.03	0.0001
Error	42	6498.866	154.735		
Corrected Total	59	17105.064			
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
Block	3	439.909	146.636	0.95	0.4263
Solution	2	275.132	137.566	0.89	0.4186
Concentration	4	2093.726	523.432	3.38	0.0174
Solution* Concentration	8	7797.430	974.679	6.30	<.0001

Table 2 Analysis of variance for root length (mm) of guayule cuttings with treatment solutions indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	97906.444	5759.203	4.36	<.0001
Error	162	213745	1319.414		
Corrected Total	179	311651.44			
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
Block	3	1313.000	437.667	0.33	0.8024
Solution	2	9160.144	4580.072	3.47	0.0334
Concentration	4	60021.889	15005.472	11.37	<.0001
Solution* Concentration	8	27411.411	3426.426	2.60	0.0107

The treatment solution and concentration showed a significant (p = 0.0186) interaction for root weight of guayule cuttings (Table 3). Indole butyric acid treatments obtained similar root weights across the concentration range, with the heaviest roots of 1.21 g and 1.14 g obtained at 30 mg/l and 120 mg/l concentrations respectively (Figure 3). Naphthalene acetamide treatment concentrations of 30 mg/l and 120 mg/l resulted in the heaviest mean root weights of 1.49 g and 1.8 g respectively. Naphthalene acetic acid treatments at 30 mg/l and 60 mg/l concentrations obtained the heaviest roots. Naphthalene acetamide treatment obtained the heaviest roots in comparison to naphthalene acetic acid and indole butyric acid (lsd = 0.726). Treatment solutions at optimum concentrations improved root weight by 0.3 g to 1.5 g, compared to the controls at 0.3 g to 0.9 g.

Table 3 Analysis of variance for root weight (g) of guayule cuttings with treatment solutions indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA)

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	37.782	2.222	2.74	0.0005
Error	162	131.489	0.812		
Corrected Total	179	169.271			
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
Block	3	8.41	2.803	3.45	0.0179
Solution	2	1.336	0.668	0.82	0.4410
Concentration	4	12.551	3.138	3.87	0.0050
Solution* Concentration	8	15.486	1.936	2.38	0.0186

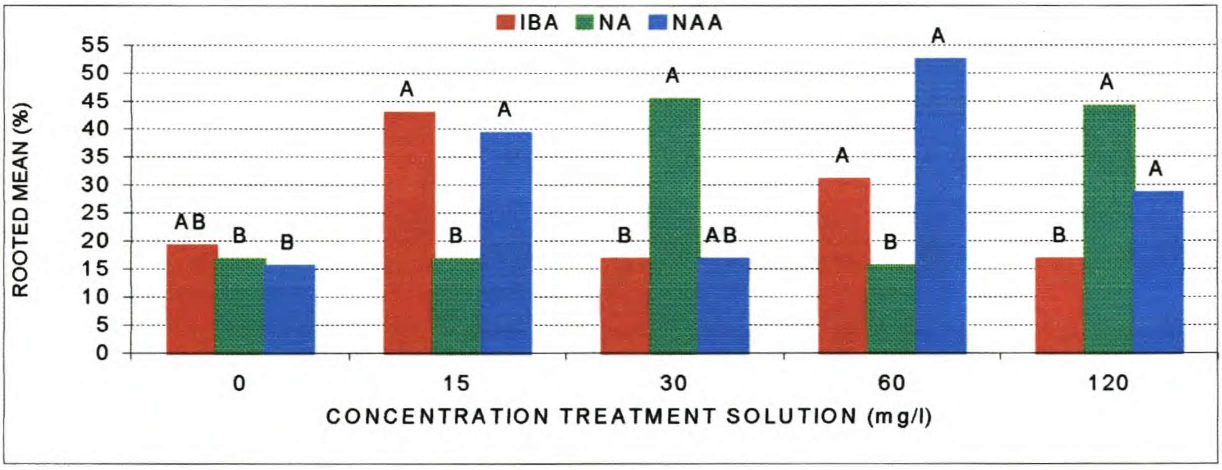

Figure 1 Rooting (%) response of guayule cuttings to specific concentrations of indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA) treatment solutions. (lsd = 17.751). Different letters indicate significant differences at p = 0.05 for each treatment solution.

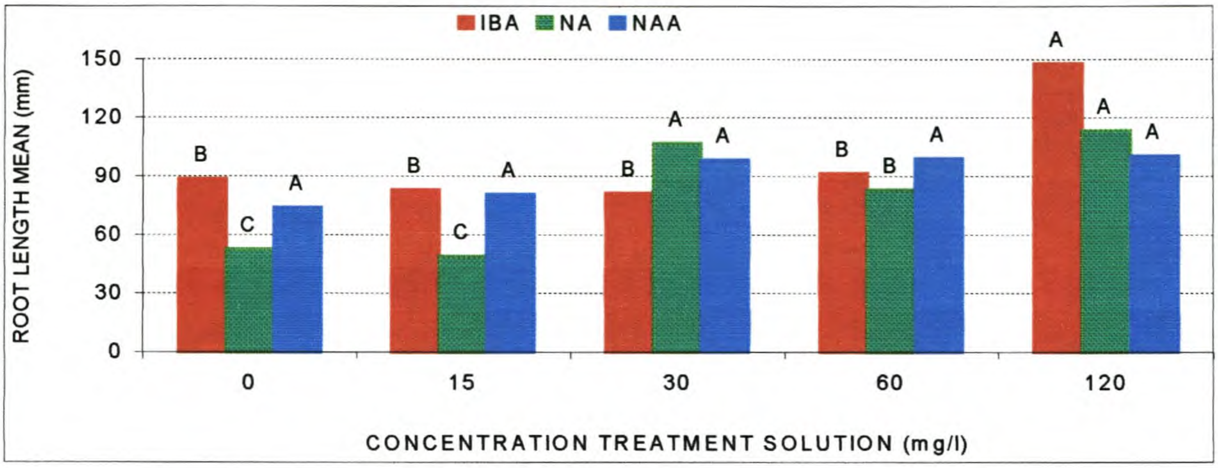

Figure 2 Root length (mm) produced by guayule cuttings with specific concentrations of indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA) treatment solutions. (lsd = 29.283). Different letters indicate significant differences at p = 0.05 for each treatment solution.

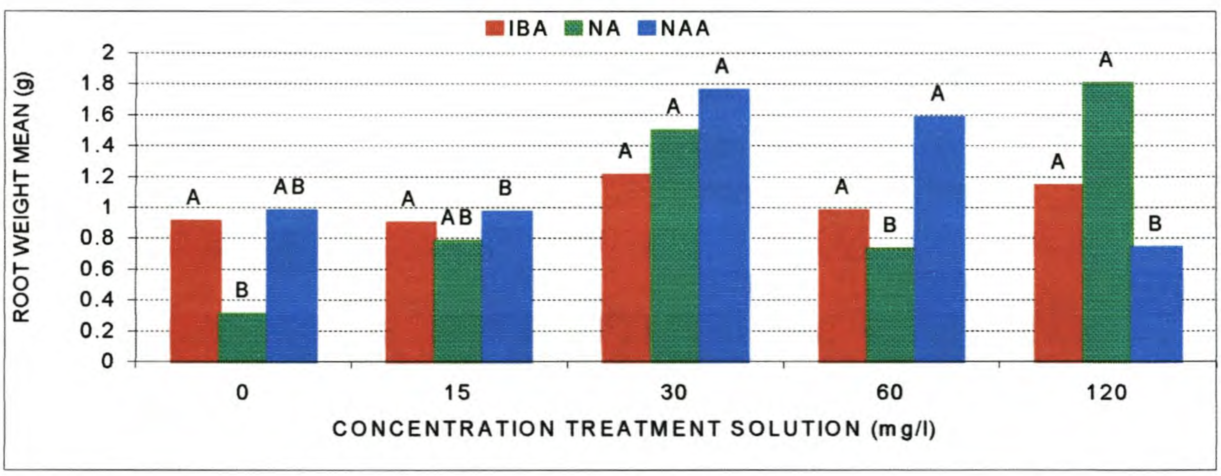

Figure 3 Root weight (g) of guayule cuttings obtained with specific concentrations of indole butyric acid (IBA), naphthalene acetamide (NA) and naphthalene acetic acid (NAA) treatment solutions. (lsd = 0.726). Different letters indicate significant differences at p = 0.05 for each treatment solution.

Discussion

Naphthalene acetic acid treatment rooted more cuttings than indole butyric acid and naphthalene acetamide, with optimum rooting response of 52.38% at a concentration of 60 mg/l. Indole butyric acid treatment produced the longest roots of 147.83 mm at 120 mg/l concentration, though roots were thin and stringy. Naphthalene acetamide obtained the best root weight of 1.8 g at an optimum concentration of 120 mg/l. Applications of treatment solutions indicated specific concentrations for optimum rooting responses, such that naphthalene acetic acid at 60 mg/l improved root formation (by 30%), indole butyric acid at 120 ppm improved root length (by 50 mm) and naphthalene acetamide at 120 mg/l improved root weight (by 1.5 g), when compared to the controls. The positive rooting response of guayule cuttings to indole butyric acid treatment have also been reported by Smith (1944), but with additional aeration of water at 25^{0}C root development resulting in 80% of material in 17 days. Nishimura *et al.* (1944) also confirmed improved rooting of cuttings with indole butyric acid (producing long roots) and naphthalene acetamide (producing thick roots) and reported 95% rooting in two to three weeks. Lloyd (1911) however could not induce rooting of guayule stems that had no root tissues present, making some reference to the variability of mother-plant material that is common due to inherent quality characteristics resulting from asexual reproduction (Stewart & Henderson, 1986). The guayule plant naturally lends itself to further improvement by vegetative procedures due to its physiological character. In the field it naturally produces adventitious roots from branches that are separated from the mother-plant. Also, it has an innate persistence to survive that was obvious from the leaf sprouting and flower budding of the cuttings in the nursery environment. Though rooting improvements in this experiment have been relatively small, further development of vegetative propagation techniques to increase rooting results are required to successfully multiply selected cultivars with elite qualities of vigorous growth and high latex yield.

Acknowledgements

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PART 3 GUAYULE FIELD ESTABLISHMENT

CHAPTER VI GROWTH POTENTIAL AND BIOMASS PRODUCTION OF FIELD ESTABLISHED GUAYULE CULTIVARS

Abstract

The growth potential and biomass production of guayule cultivars were investigated at different areas and under different environmental conditions in South Africa. Trial plots were 10x10 m, rows 1 m apart and 30 cm between plants, and each cultivar (10 plants per unit) placed at random and replicated 6 times. Plantings were evaluated as a dryland practice, though irrigation was supplied only for establishment. Growth (stand count, height, canopy diameter, stem diameter) and biomass (wet and dry weight) were recorded for (1) one-year old plantings established in April 2001 at Elsenburg, Graaff-Reinet and Oudtshoorn, and (2) six-month old plantings established in October 2001 at Bethulie, Glen and Upington. Analysis of variance was done to determine mean growth and biomass for the different areas and cultivars. (1) There were significant interactions between the factors area and cultivar for stand count and height, while canopy diameter and stem diameter differences were significant only within factors. The greatest growth potential was produced by cultivars AZ-2 and AZ-3, and Oudtshoorn was the best area for growth potential and biomass production. (2) Interaction between area and cultivar was significant for plant height, but were not significant for stand count, canopy diameter and stem diameter. Cultivars produced similar results for biomass production, but were significantly different in the different areas of Bethulie, Glen and Upington. Growth potential and biomass production of guayule was influenced by the availability of water during the growth of the plant.

Keywords: biomass production, field trials, growth potential, guayule, *Parthenium argentatum*

Introduction

Guayule is a source of natural rubber that is non-allergenic to human tissues (Cornish & Siler, 1996). The rubber strength, elasticity and viral impermeability is useful in applications where durability is required and disease transmission needs to be limited, such as condoms and catheters (Cornish & Siler, 1996). The plant also produces resin that can be used in paints, adhesives and varnishes (Campos-Lopez & Anderson, 1983).

Guayule originates from the arid regions of north-central Mexico (in Coahuila, Durango, Zacatecas, San Luis Potosi and Neuvo Leon), and south (the Big Bend areas) and south-west of Texas (in Presidio, Brewster and Pecos Counties). The plant is distributed at an altitude of 600-3000 meters above sea level and survive on annual rainfall of 250-380 mm. It occurs on well-drained calcareous soils with a pH of 6-8, in areas with temperature ranging between $0^{O}C$ and $35^{O}C$ (Lloyd, 1911).

Geographical regions in South Africa (Figure 1) with potential for guayule cultivation are selected in comparison with the native habitat of the plant. Suitable soil structures of red sands or sandy loams on limestone underlay occur east of the Kalahari. The north-central, north-eastern and eastern regions of South Africa also have suitable medium to high fertile soils. South Africa has predominant winter and summer rainfall patterns generally occurring from south-west to north-east of the country. Adequate rainfall of 200-600 mm *per annum* occur in a band from the Western Cape, across the Eastern Cape, Free State and North-West, to the Northern Province (Smit, 1989; Milthorpe *et al.,* 1991).

In its natural habitat guayule grows vegetatively in summer and produce latex at the start of winter (Lloyd, 1911; Hammond & Polhamus, 1965). The latex is found in all plant structures, though the greatest concentration occurs 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 accumulation of latex in the plant allows the plant to survive extreme cold conditions.

Establishment of guayule requires mechanical blade ploughing (15-20cm), disc turning and soil leveling. This promotes good drainage and optimal root penetration during establishment since transplanted seedlings are prone to initial drowning. Trial plantings were evaluated as a dryland practice, though irrigation was supplied for one month only from the date of planting. No fertilization was supplemented to the soil since there is no proof that related costs can be justified in relation to the latex product (Ferraris, 1986). Since guayule is a slow grower, regular weeding prevents weed competition for available growing space and soil moisture. The main disease threat on guayule is from fungi (*Verticillium albo-arum, Phymatotrichum omnivorum)* in the soil causing wilting and root rot (Hammond & Polhamus, 1965).

In order to determine the growth potential and biomass production of guayule cultivars trial plots were established at (1) Elsenburg (Western Cape), Oudtshoorn (Western Cape) and Graaff-Reinet (Eastern Cape), and (2) at Upington (Northern Cape), Glen (Free State) and Bethulie (Free State). Results on growth (stand count, height, canopy diameter and stem diameter) and biomass (wet and dry weight) are presented for the different cultivars and different areas.

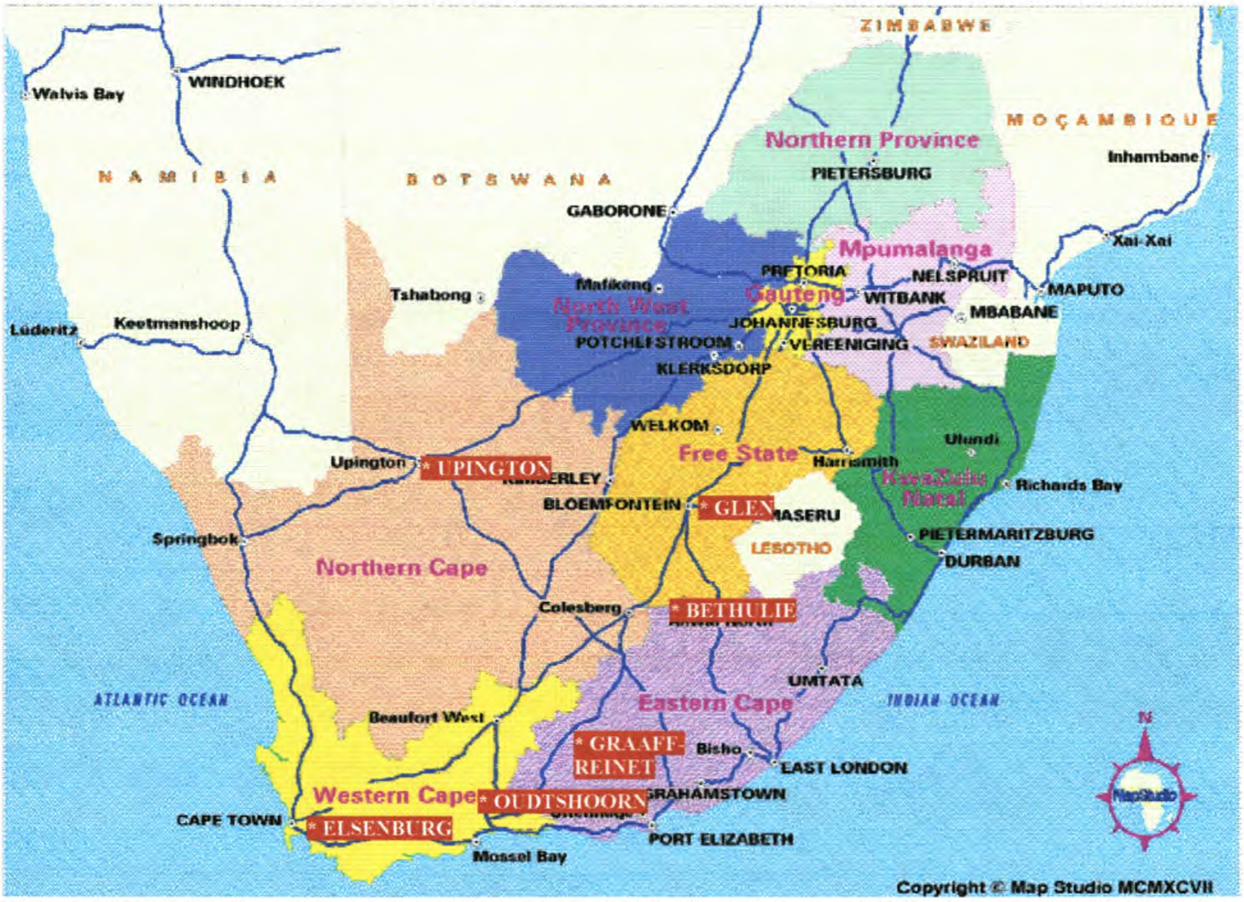

Figure 1 Distribution of guayule trial plots established at Elsenburg (Western Cape), Oudtshoorn (Western Cape), Graaff-Reinet (Eastern Cape), Upington (Northern Cape), Glen (Free State) and Bethulie (Free State) in South Africa (Map Studio).

Materials and methods

Experiment 1

In January 2001, seeds of guayule cultivars AZ-2, AZ-3, N565 and 11591 were planted in speedling trays (7x7) in a growth mixture [river sand: peat moss: polystyrene chips (2:1:1)] and placed on heated beds (±25°C) and maintained in the nursery under sprinkle irrigation (1min each hour from 7:00 to 19:00). A standard hydroponic feeding solution (Hydropon®) was supplied twice a week on alternate days until seedlings were 100 days old. Seedlings were then transferred to 50% shade for one week in preparation for field planting. Land was prepared for planting by blade ploughing (15-20cm), disc turning and soil leveling or by manual labour. Trial plots were established in April 2001 at Elsenburg (±34^{0}S; 19^{0}E), Oudtshoorn (±33.5^{0}S; 22.5^{0}E) and Graaff-Reinet (±32.5^{0}S; 24.5^{0}E). Trial plots were 10x10m, rows spaced 1m apart and 30cm between plants, and each cultivar (10 plants per unit) was placed at random and replicated 6 times. The plantings were evaluated as a dryland practice, though irrigation was supplied for one month only from the date of planting.

Experiment 2

In July 2001, seeds of guayule cultivar 11591 was not available, so only cultivars AZ-2, AZ-3 and N565 were prepared as per experiment 1 for field planting in October 2001 at Bethulie (±30.5^{0}S; 26^{0}E), Glen (±29.5^{0}S; 26.5^{0}E) and Upington (±28.5^{0}S; 21^{0}E).

Growth potential (stand count, height, canopy diameter and stem diameter) of plants was recorded every two months and biomass (wet and dry weight) production was recorded in April 2002 for one-year old plantings at Elsenburg, Oudtshoorn and Graaff-Reinet, and six-month old plantings at Bethulie, Glen and Upington. Analysis of variance was done to determine the mean growth and biomass production of the plants in the different areas and for the different cultivars (SAS, 1999).

Results

Table 1 Analyses of variance for stand count, height, canopy diameter and stem diameter of guayule cultivars (AZ-2, AZ-3, N565, 11591) in different areas (Elsenburg, Graaff-Reinet, Oudtshoorn)

Dependent Variable: Stand Count					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	28851.67	14425.83	6.84	0.0078
CULTIVAR	3	13360.00	4453.33	7.29	0.0004
AREA*CULTIVAR	6	9881.67	1646.94	2.70	0.0254
Dependent Variable: Height					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	969358.47	484679.23	52.35	<.0001
CULTIVAR	3	236480.09	78826.70	14.11	<.0001
AREA*CULTIVAR	6	101762.31	16960.39	3.04	0.0140
Dependent Variable: Canopy Diameter					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	718348.54	359174.27	44.19	<.0001
CULTIVAR	3	123592.92	41197.64	12.02	<.0001
AREA*CULTIVAR	6	18093.91	3015.65	0.88	0.5176
Dependent Variable: Stem Diameter					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	652.02	326.01	84.13	<.0001
CULTIVAR	3	140.63	46.88	11.92	<.0001
AREA*CULTIVAR	6	29.84	4.97	1.26	0.2926

There were significant interaction between area and cultivar for stand count ($p = 0.0254$) and height ($p = 0.0140$) (Table 1). Guayule cultivars AZ-2, AZ-3, N565 and 11591 responded differently for stand count and height in the areas Elsenburg, Graaff-Reinet and Oudtshoorn (Figure 2a and 2b). Canopy diameter and stem diameter had significant differences within factors, but had no significant interaction between area and cultivar (Table 1, Figure 2c and 2d).

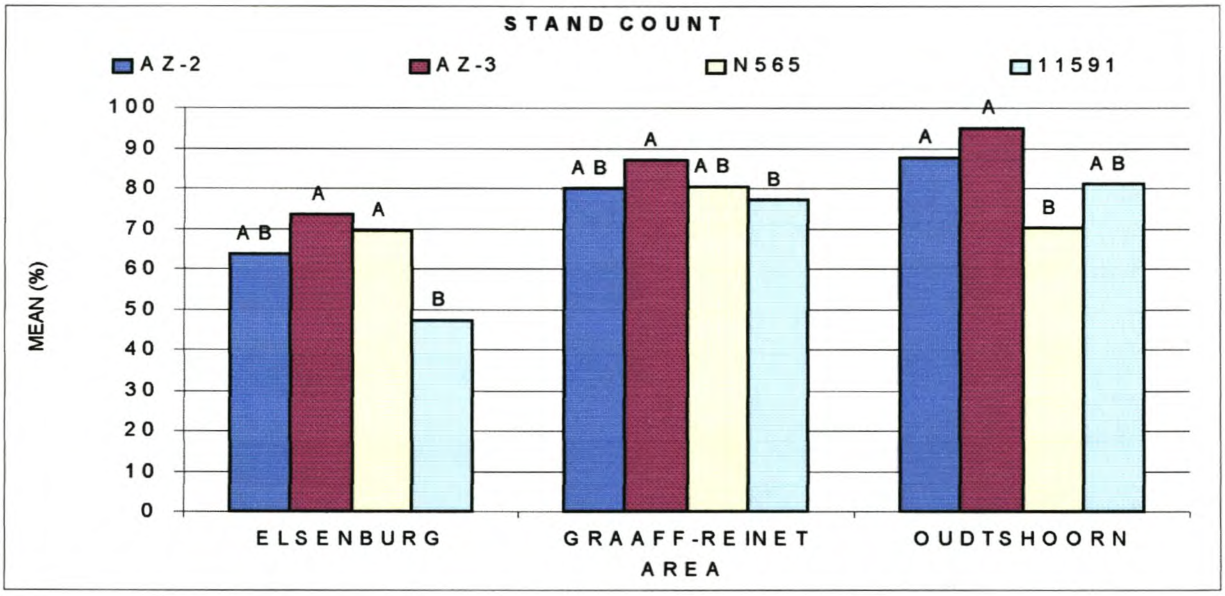

Figure 2a Mean stand count of guayule cultivars (AZ-2, AZ-3, N565, 11591). Different letters indicate significant differences at p = 0.05 within areas. Least significant difference (lsd) for area = 12.641 and lsd for cultivar = 7.4207.

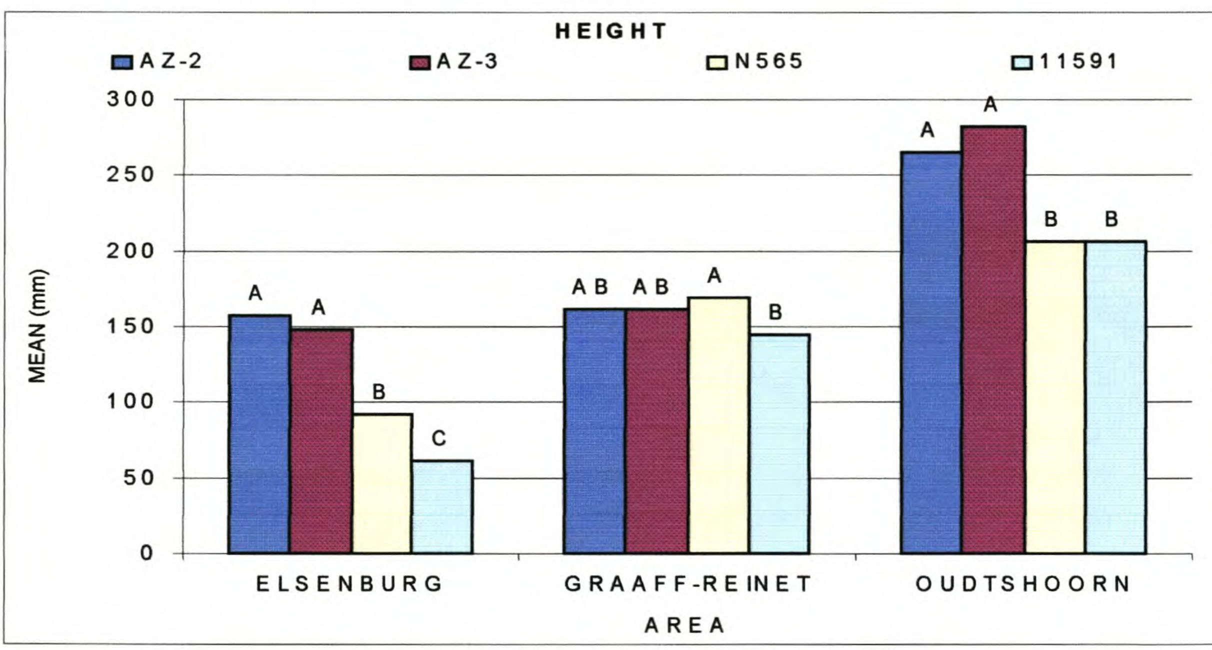

Figure 2b Mean height of guayule cultivars (AZ-2, AZ-3, N565, 11591). Different letters indicate significant differences at p = 0.05 within areas. Least significant difference (lsd) for area = 26.477 and lsd for cultivar = 22.442.

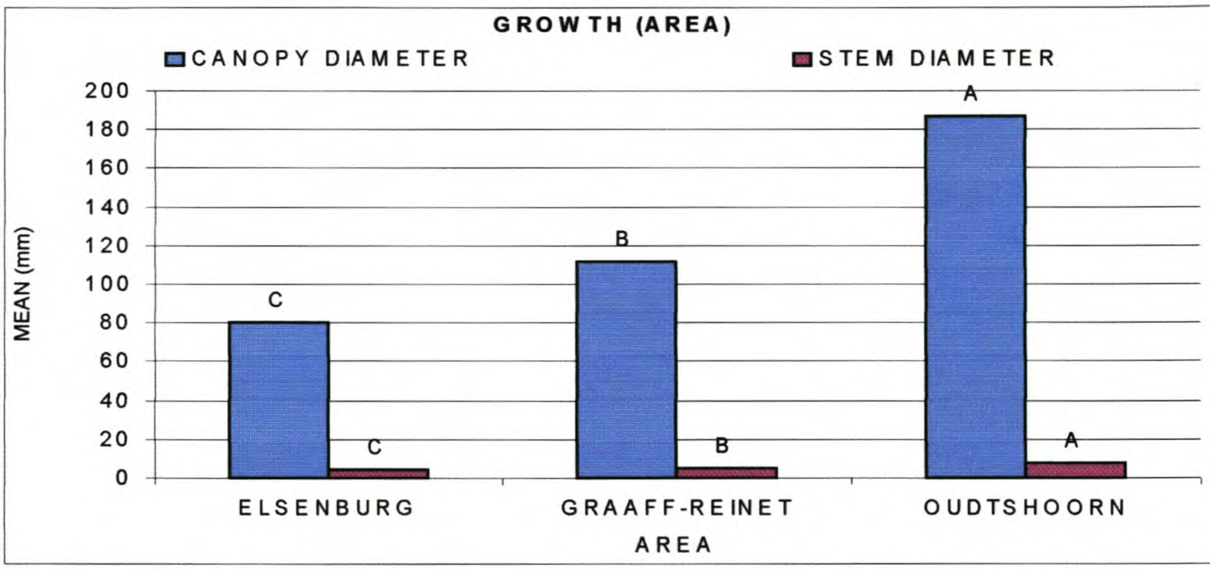

Figure 2c Mean canopy and stem diameter of guayule cultivars (AZ-2, AZ-3, N565, 11591) in different areas. Different letters indicate significant differences at p = 0.05 between areas. Area least significant difference (lsd) for canopy diameter = 24.807 and stem diameter = 0.5417.

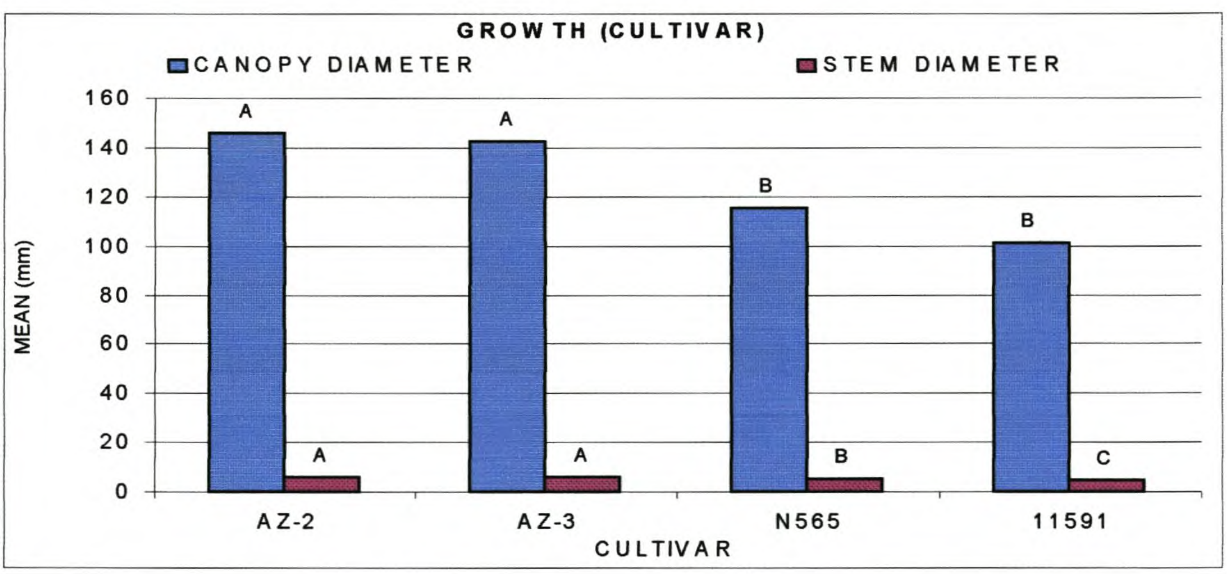

Figure 2d Mean canopy and stem diameter for different guayule cultivars (AZ-2, AZ-3, N565, 11591). Different letters indicate significant differences at p = 0.05 between cultivars. Cultivar least significant difference (lsd) for canopy diameter = 17.58 and stem diameter = 0.5954.

Table 2 Analyses of variance for dry weight and wet weight of guayule cultivars (AZ-2, AZ-3, N565, 11591) in different areas (Elsenburg, Graaff-Reinet, Oudtshoorn)

Dependent Variable: Dry Weight					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
BLOCK	5	1303.05	260.61	0.58	0.7127
CULTIVAR	3	13980.51	4660.17	10.43	<.0001
AREA	2	43005.22	21502.61	48.11	<.0001
AREA*CULTIVAR	6	12976.03	2162.67	4.84	0.0005
Dependent Variable: Wet Weight					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
BLOCK	5	3165.86	633.17	0.45	0.8147
CULTIVAR	3	37842.06	12614.02	8.88	<.0001
AREA	2	95498.06	47749.03	33.60	<.0001
AREA*CULTIVAR	6	27413.24	4568.87	3.22	0.0089

There were significant interaction between area and cultivar for dry weight (p = 0.0005) and wet weight (p = 0.0089) (Table 2). Results for biomass production (dry weight and wet weight) were significantly different for the cultivars in the different areas (Figure 3a and 3b).

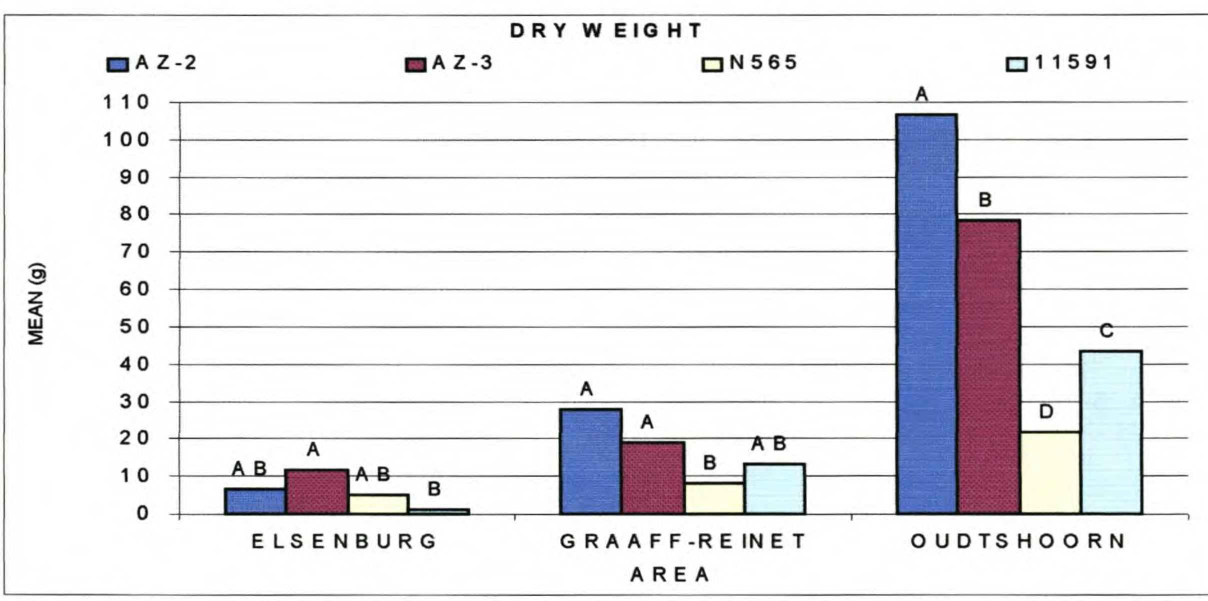

Figure 3a Mean dry weight for different guayule cultivars (AZ-2, AZ-3, N565, 11591) in different areas. Different letters indicate significant differences at p = 0.05 within areas. Least significant difference (lsd) for area = 12.23 and lsd for cultivar = 14.122.

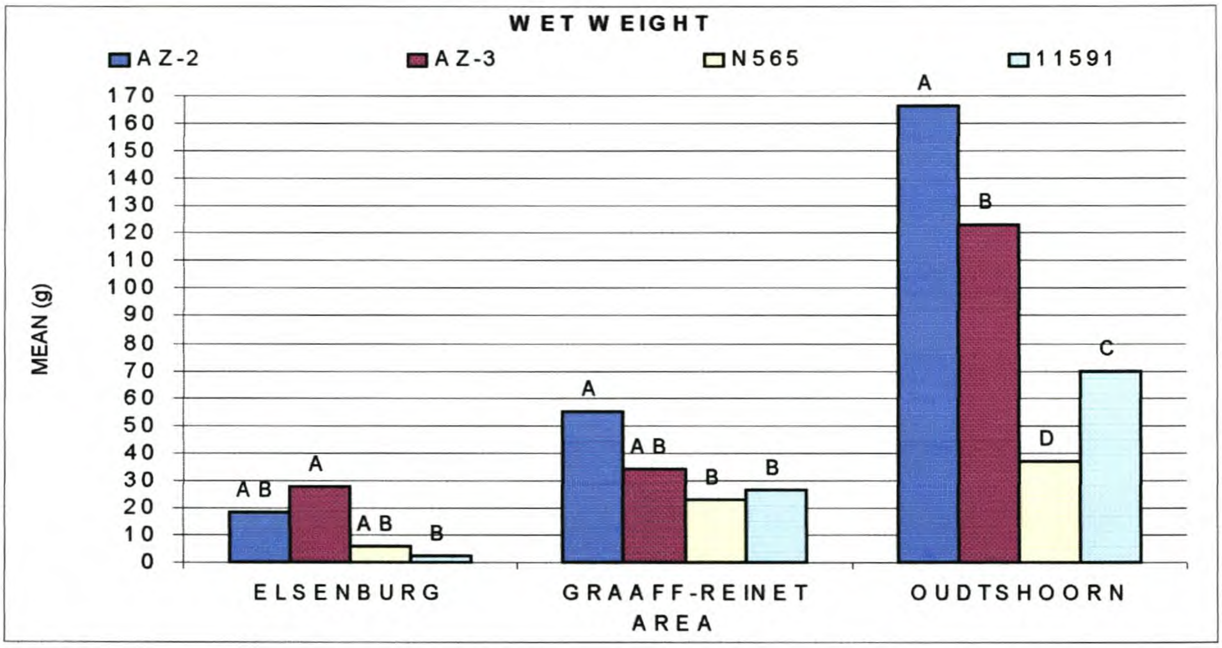

Figure 3b Mean wet weight for different guayule cultivars (AZ-2, AZ-3, N565, 11591) in different areas. Different letters indicate significant differences at p = 0.05 within areas. Least significant difference (lsd) for area = 21.808 and lsd for cultivar = 25.182.

The soil data show similar loam soil structures for Elsenburg, Graaff-Reinet and Oudtshoorn. The physical effort of digging revealed tighter compacted soils at Elsenburg and Graaff-Reinet compared to the less compacted soils at Oudtshoorn. The pH of the soil in the different areas range between 4 and 8, which are acceptable levels for the growth of guayule (Table 5).

Elsenburg had rainfall well above 50 mm in May (103.9 mm), July (309 mm), August (212.1 mm), September (120.1 mm) and January (173.3 mm), with an average temperature range between 10^0C and 30^0C (Figure 4a). Graaff-Reinet rainfall range between 50 and 100 mm for April (92.9 mm, November (76.9 mm), December (93 mm) January (83.3 mm) and March (67 mm) (Figure 4b). Average temperature range between 10^0C and 30^0C, with a minimum below 10^0C from April 2001 to October. Oudtshoorn mostly had rainfall below 15 mm, but had an increase to 40.4 mm in October, with a peak of 73.7 in November and a rise to 31.5 mm in February (Figure 4c). Average temperature range between 10^0C and 30^0C, with a decrease well below 10^0C in June, July and August.

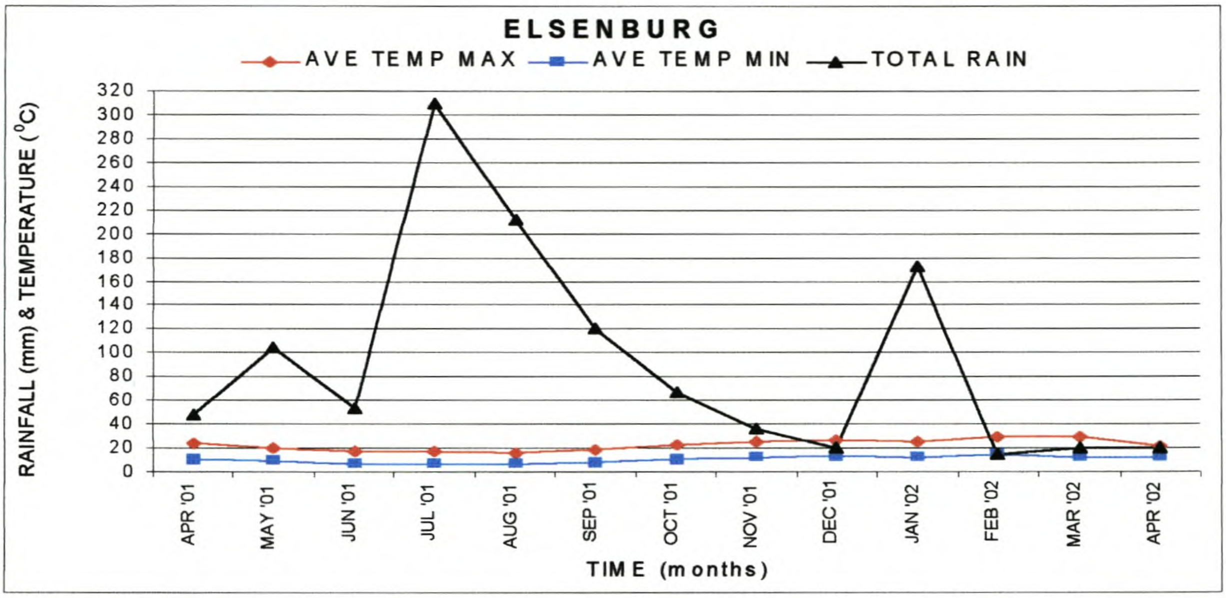

Figure 4a Rainfall pattern and temperature regime of guayule trial plot at Elsenburg.

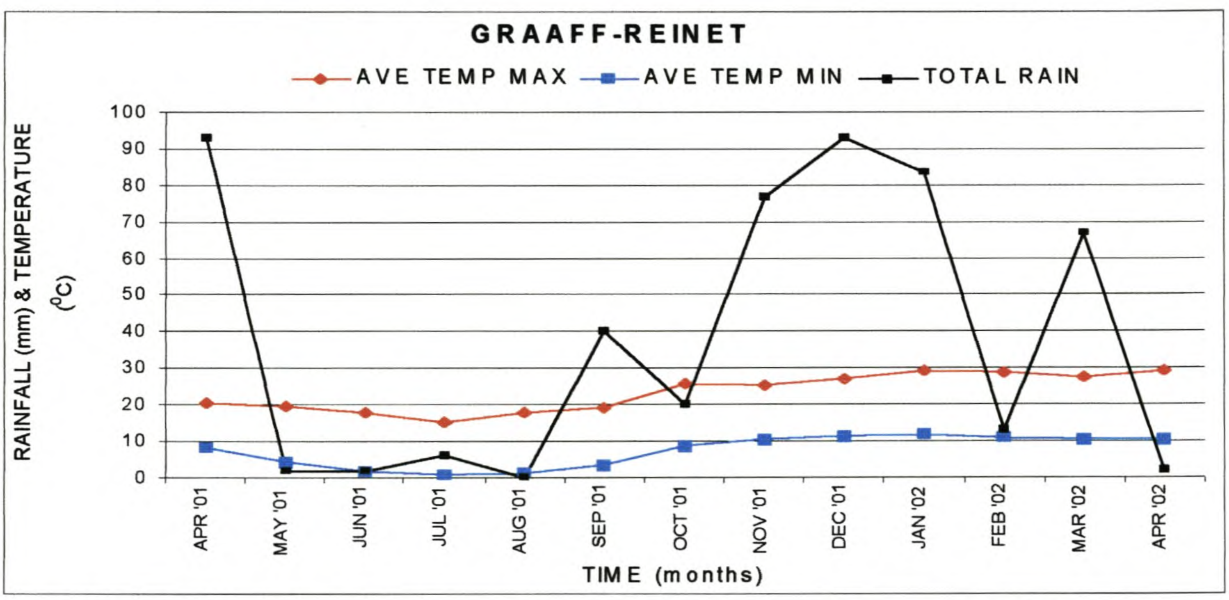

Figure 4b Rainfall pattern and temperature regime of guayule trial plot at Graaff-Reinet.

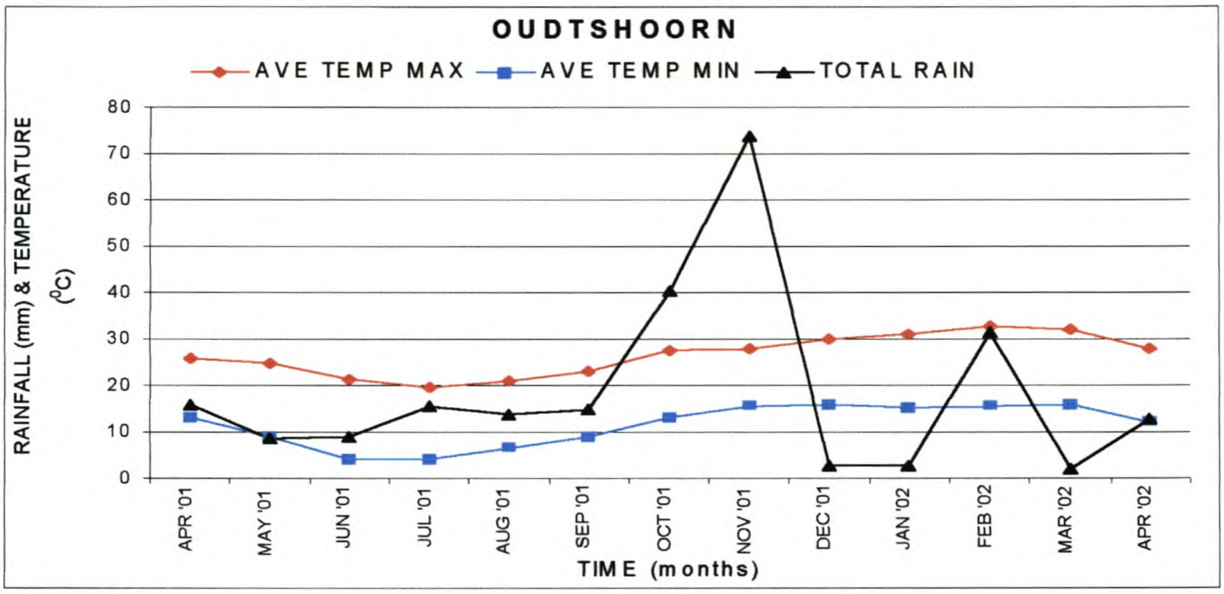

Figure 4c Rainfall pattern and temperature regime of guayule trial plot at Oudtshoorn.

Table 5 Soil analysis of guayule trial plots at Elsenburg, Graaff-Reinet and Oudtshoorn

	Soil structure	pH (KCL)	Resistance (ohms)	Sodium (Na) (mg/kg)	Phosphor (P) (mg/kg)	Potassium (K) (mg/kg)	Calcium (Ca) (me%)	Magnesium (Mg) (me%)
ELSENBURG (30cm)	loam	5	2300	20	65	57	0.94	0.31
ELSENBURG (60cm)	loam	4.7	3940	16	48	32	0.77	0.23
GRAAFF-REINET (30cm)	loam	8	930	139	627	895	22.3	10.57
GRAAFF-REINET (60cm)	loam	7.8	1100	177	432	545	16.71	8.18
OUDTSHOORN (30cm)	loam	8.2	410	306	124	525	9.59	4.17
OUDTSHOORN (60cm)	loam	8.3	540	328	129	290	8.5	7.78

Table 3 Analyses of variance for stand count, height, canopy diameter and stem diameter of guayule cultivars (AZ-2, AZ-3, N565) in different areas (Bethulie, Glen, Upington)

Dependent Variable: Stand Count					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	4668.52	2334.26	2.15	0.1514
CULTIVAR	2	235.19	117.59	0.27	0.7617
AREA*CULTIVAR	4	3720.37	930.09	2.17	0.0962
Dependent Variable: Height					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	874129.17	437064.58	103.91	<.0001
CULTIVAR	2	3063.72	1531.86	0.31	0.7345
AREA*CULTIVAR	4	70676.44	17669.11	3.60	0.0165
Dependent Variable: Canopy Diameter					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	604897.56	302448.78	28.28	<.0001
CULTIVAR	2	14588.67	2794.33	0.81	0.4550
AREA*CULTIVAR	4	80558.11	20139.53	2.23	0.0891
Dependent Variable: Stem Diameter					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
AREA	2	524.96	262.48	18.29	<.0001
CULTIVAR	2	2.80	1.40	0.24	0.7874
AREA*CULTIVAR	4	14.43	3.61	0.62	0.6508

There were significant interaction between area and cultivar for height (p = 0.0165) (Table 3). Guayule cultivars AZ-2, AZ-3, N565 and 11591 responded differently for height in the areas Bethulie, Glen and Upington (Figure 5a). Stand count, canopy diameter and stem diameter had no significant interaction between area and cultivar (Table 3, Figure 5b, 5c, 5d and 5e). There were no significant differences between cultivars for stand count, canopy diameter and stem diameter. Canopy diameter and stem diameter were however significantly different in the different areas.

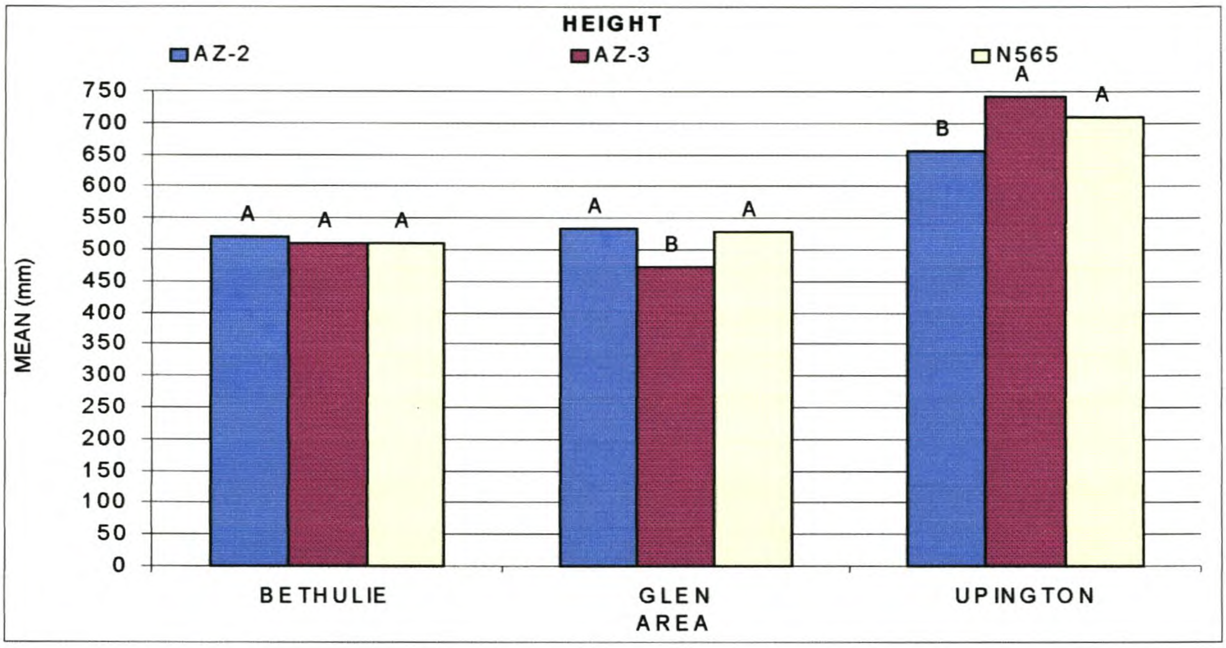

Figure 5a Mean height of guayule cultivars (AZ-2, AZ-3, N565) in different areas. Different letters indicate significant differences at p = 0.05 within areas. Least significant difference (lsd) for area = 32.583 and lsd for cultivar = 33.746.

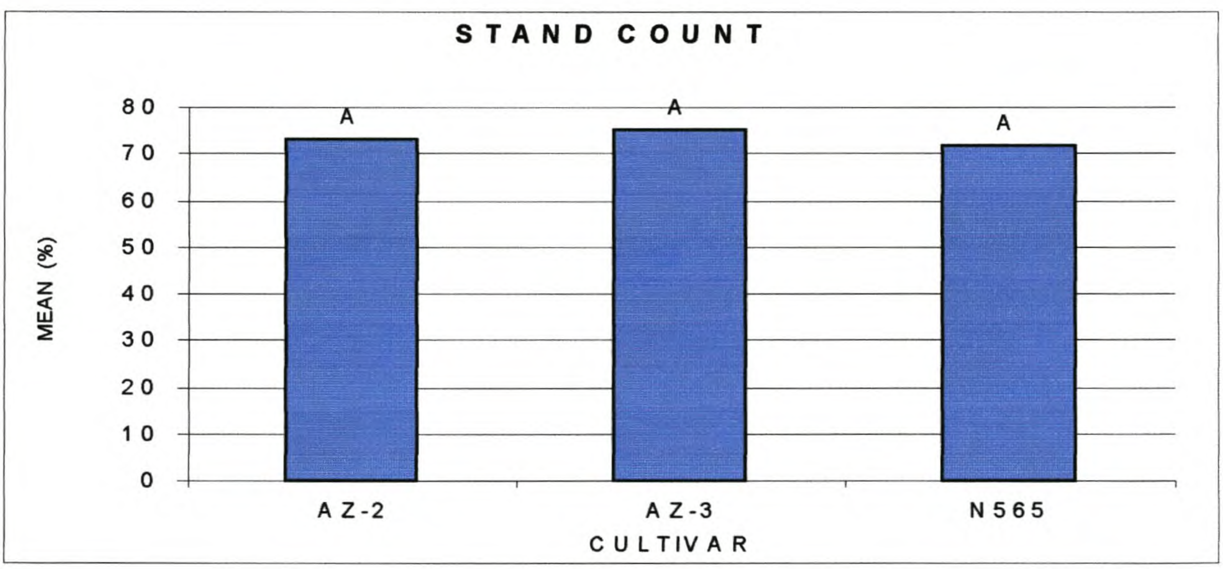

Figure 5b Mean stand count of different guayule cultivars (AZ-2, AZ-3, N565). Different letters indicate significant differences at p = 0.05 between cultivars. Least significant difference (lsd) for cultivar = 9.9603.

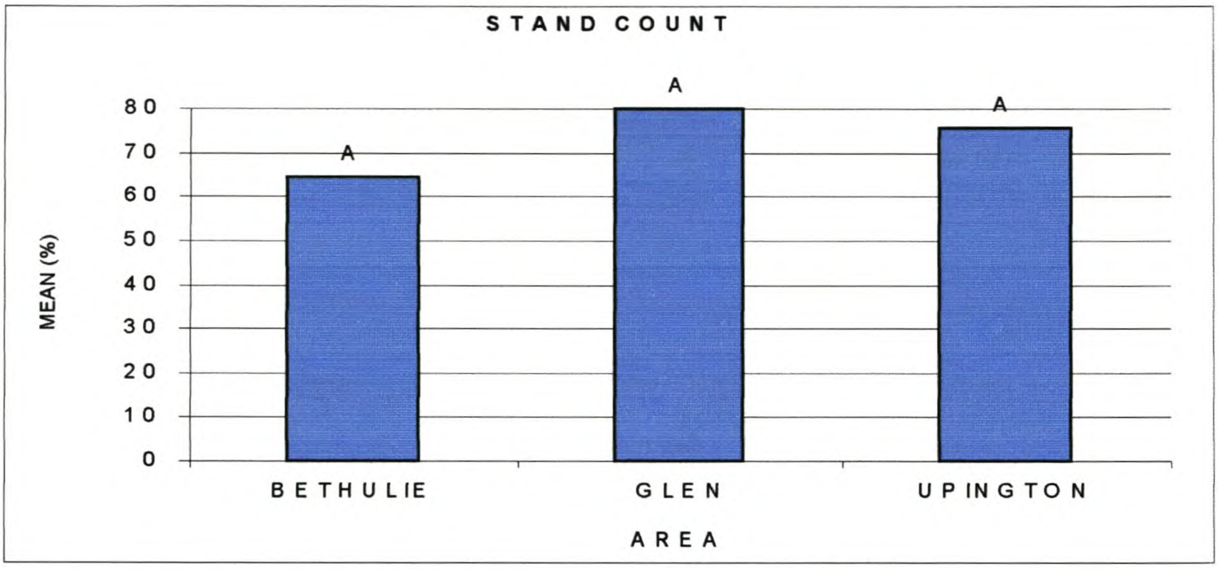

Figure 5c Mean stand count of guayule cultivars (AZ-2, AZ-3, N565) in different areas. Different letters indicate significant differences at p = 0.05 between areas. Least significant difference (lsd) for area = 16.568.

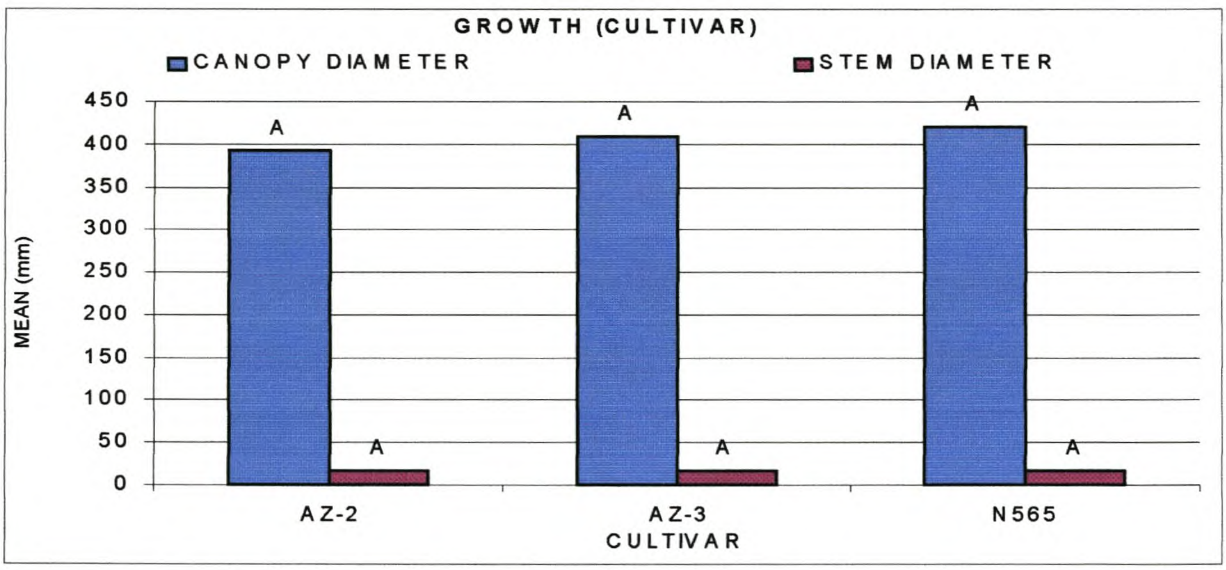

Figure 5d Mean canopy and stem diameter of different guayule cultivars (AZ-2, AZ-3, N565). Different letters indicate significant differences at p = 0.05 between cultivars. Cultivar least significant difference (lsd) for canopy diameter = 45.72 and stem diameter = 1.1597.

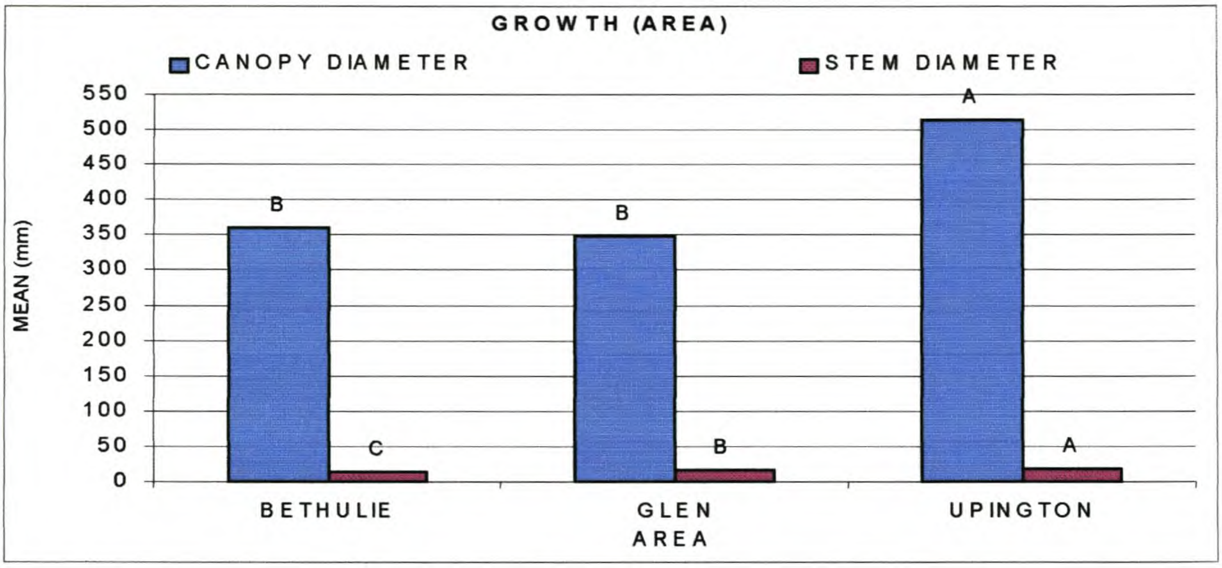

Figure 5e Mean canopy and stem diameter of guayule cultivars (AZ-2, AZ-3, N565) in different areas. Different letters indicate significant differences at p = 0.05 between areas. Area least significant difference (lsd) for canopy diameter = 51.952 and stem diameter = 1.903.

Table 4 Analyses of variance for dry weight and wet weight of guayule cultivars (AZ-2, AZ-3, N565) in different areas (Bethulie, Glen, Upington)

Dependent Variable: Dry Weight					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
BLOCK	5	15043.05	3008.61	0.37	0.8671
CULTIVAR	2	11105.62	5552.81	0.68	0.5123
AREA	2	574543.24	287271.62	35.19	<.0001
AREA*CULTIVAR	4	38871.75	9717.94	1.19	0.3298
Dependent Variable: Wet Weight					
Source	Degrees of Freedom	Type I SS	Mean Square	F Value	Pr > F
BLOCK	5	62146.20	12429.24	0.42	0.8296
CULTIVAR	2	24156.05	12078.02	0.41	0.6654
AREA	2	3236504.6	1618252.28	55.13	<.0001
AREA*CULTIVAR	4	288883.17	72220.79	2.46	0.0608

There were no significant interaction between area and cultivar for dry weight (p = 0.3298) and wet weight (p = 0.0608) (Table 4). Results for biomass production (dry weight and wet weight) were significantly different in the different areas, but there were no significant differences between cultivars (Figure 6a and 6b).

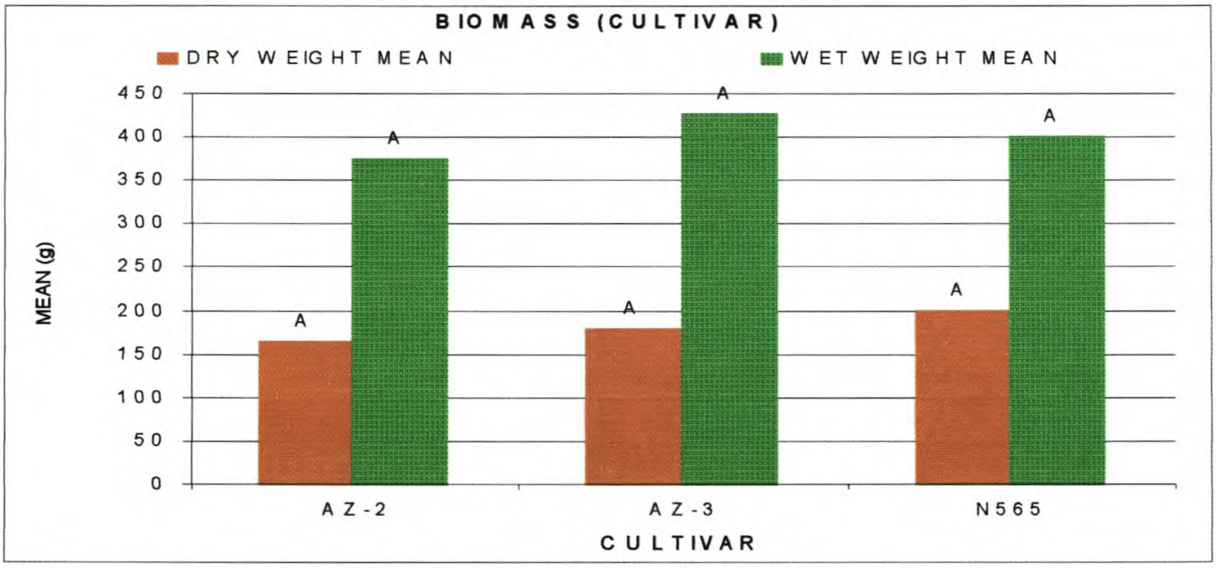

Figure 6a Mean biomass (dry weight, wet weight) of guayule cultivars (AZ-2, AZ-3, N565). Different letters indicate significant differences at p = 0.05 between cultivars. Cultivar least significant difference (lsd) for dry weight = 60.868 and wet weight = 115.42.

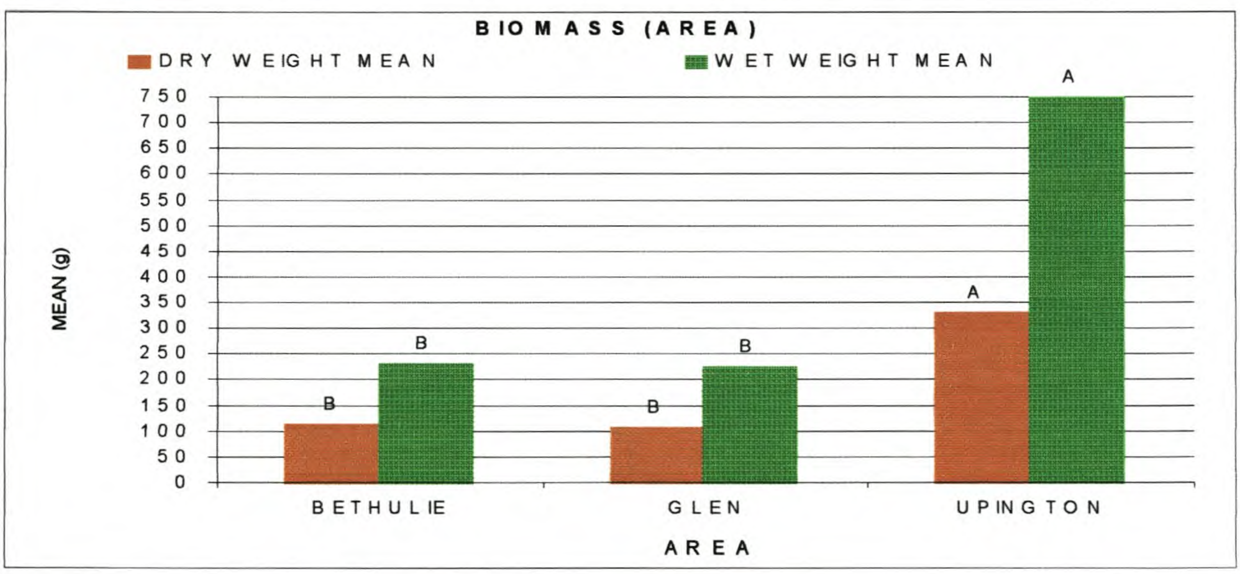

Figure 6b Mean biomass (dry weight, wet weight) of guayule cultivars (AZ-2, AZ-3, N565) in different areas. Different letters indicate significant differences at p = 0.05 between areas. Area least significant difference (lsd) for dry weight = 60.868 and wet weight = 115.42.

Similar loam soil structures were found at Bethulie and Glen, with the exception of Upington with its sandy loam soil. Tighter compacted soils were found at Glen compared to the less compacted soils at Bethulie, and the even lighter soils at Upington, judging from the physical effort of digging. The different areas had a soil pH range between 4 and 8 (Table 6).

Table 6 Soil analysis of guayule trial plots at Bethulie, Glen and Upington

	Soil structure	pH (KCL)	Resistance (ohms)	Sodium (Na) (mg/kg)	Phosphor (P) (mg/kg)	Potassium (K) (mg/kg)	Calcium (Ca) (me%)	Magnesium (Mg) (me%)
BETHULIE (30cm)	loam	7.4	290	54	456	764	17.5	6.96
BETHULIE (60cm)	loam	7.2	400	101	234	449	7.94	5.92
GLEN (30cm)	loam	4.8	2830	9	5	179	1.21	0.89
GLEN (60cm)	loam	4.9	2320	10	4	158	1.31	0.99
UPINGTON (30cm)	s/loam	6.6	-	33	27	205	10.53	3.92
UPINGTON (60cm)	s/loam	6.9	-	36	14	148	10.84	3.71

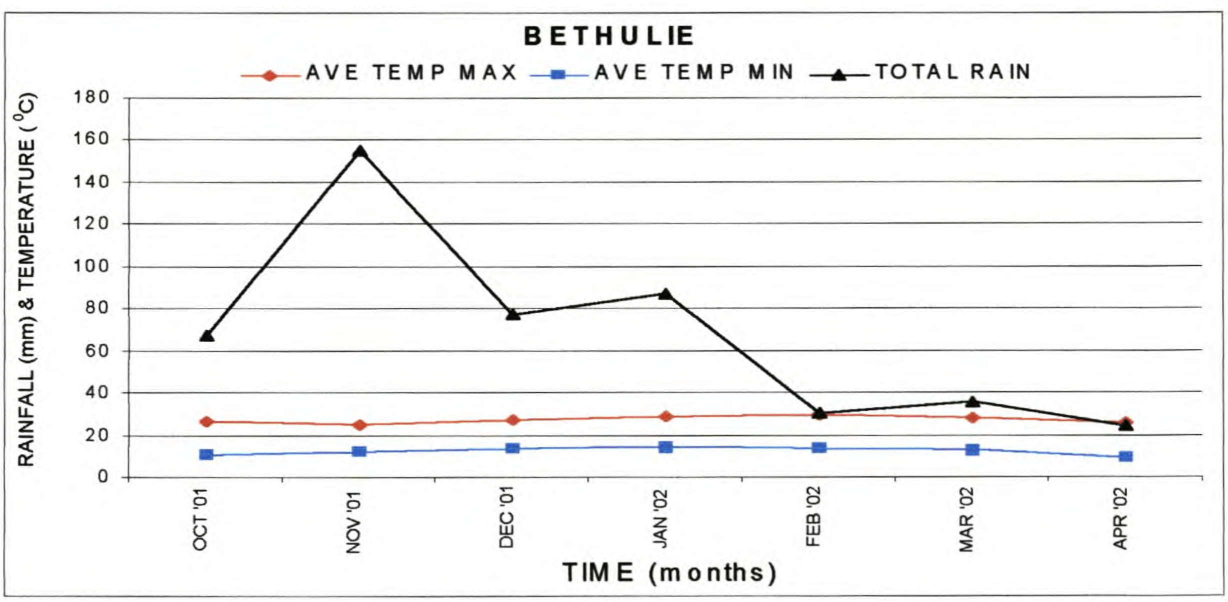

Figure 7a Rainfall pattern and temperature regime of guayule trial plot at Bethulie.

The rainfall pattern for Bethulie show a peak of 154.8 mm in November, a slight rise above 80 mm in January 2002, then a decrease to between the 20 mm and 40 mm range (Figure 7a). Average temperature for Bethulie and Glen range between 10^0C and 30^0C. Glen had peak rainfall of 145 mm in November and 151.5 mm in January, decreasing to between the 20 mm and 40 mm range (Figure 7b). Exceptional rainfall was recorded for Upington (82.5 mm in November, 32.9 mm in January, 55.8 mm in February and 57.8 mm in March) with average minimum and maximum temperature between 15 and 35^{O}C (Figure 7c).

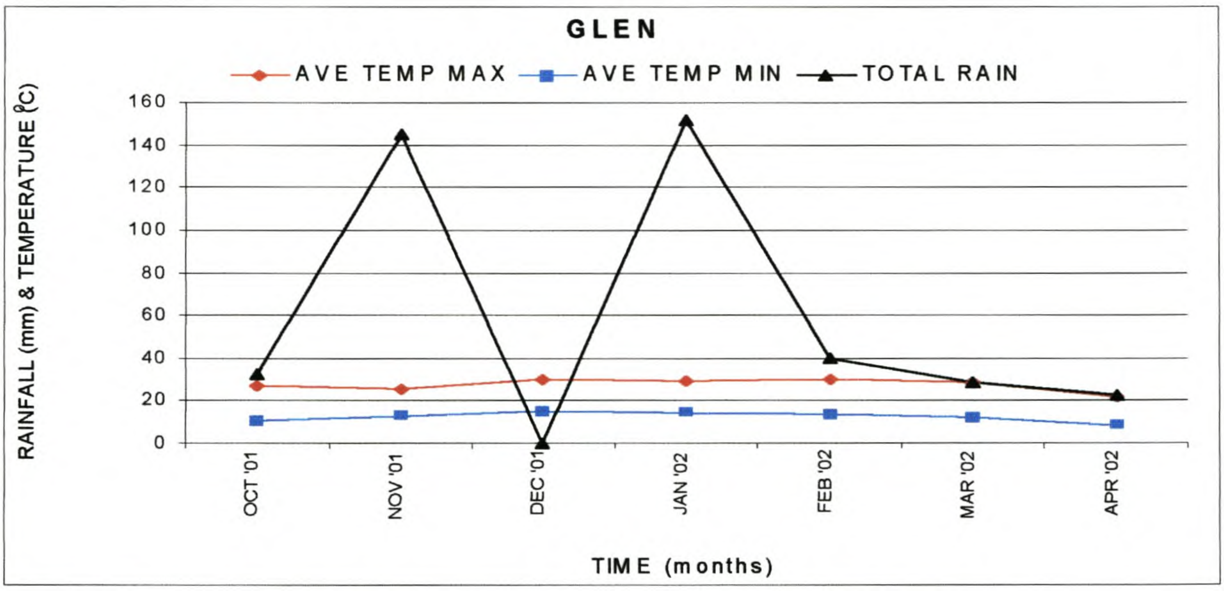

Figure 7b Rainfall pattern and temperature regime of guayule trial plot at Glen.

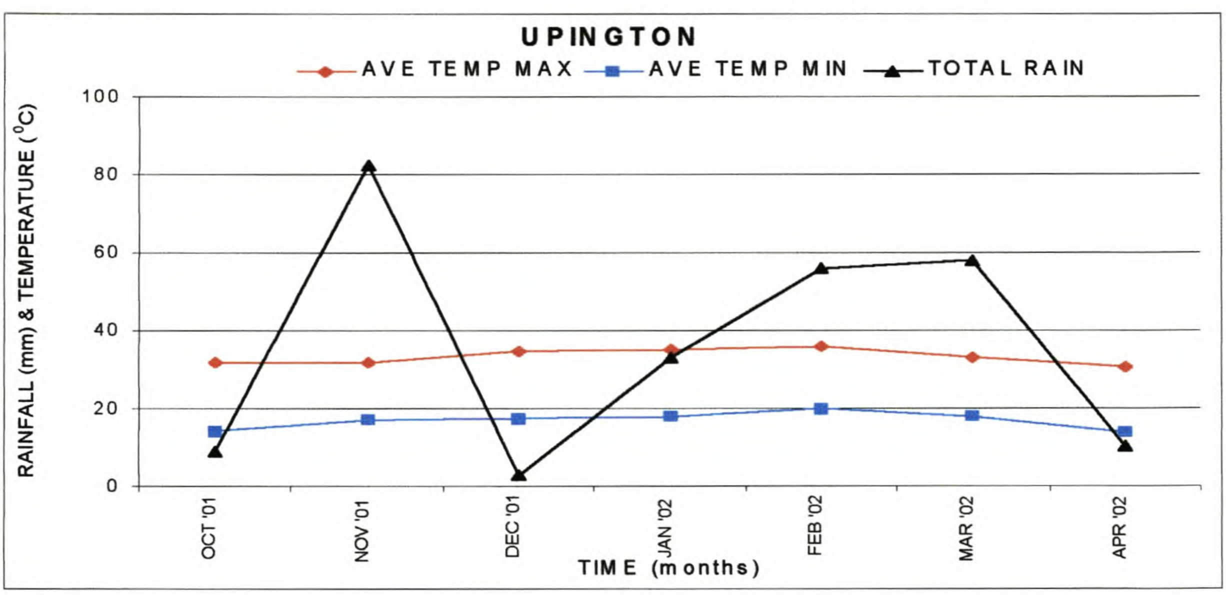

Figure 7c Rainfall pattern and temperature regime of guayule trial plot at Upington.

Discussion

Experiment 1

Field establishment evaluations show significant interaction between area and cultivar for stand count and height, while canopy diameter and stem diameter were significant only within factors. Cultivar AZ-2 and AZ-3 were able to grow better than cultivars N565 and 11591 under environmental conditions in the areas Elsenburg, Graaff-Reinet and Oudtshoorn. Oudtshoorn was the best area for growth potential and biomass production, followed by Graaff-Reinet and then Elsenburg. Biomass production also had significant interaction between area and cultivar. Cultivars AZ-2 and AZ-3 are bulkier, broad-leaved shrubs and produced the greatest biomass in comparison to the slender cultivar N565 and extremely slim-leafed cultivar 11591.

Experiment 2

Interaction between area and cultivar was significant for plant height, but were not significant for stand count, canopy diameter and stem diameter. Cultivars produced similar results for biomass production, but were significantly different in the different areas of Bethulie, Glen and Upington. Significant differences exist between the different areas such that Upington produced greater growth and biomass compared to Bethulie and Glen, where results were closely similar.

Predominantly good rains after planting in October visibly improved the growth potential of the plants at Upington, Bethulie and Glen. The poor rainfall after planting in April contributed to poorer growth results at Graaff-Reinet and Oudtshoorn. Plantings done in October had a notable advantage over those planted in April due to the prevailing rainfall during establishment and resulted in improved vegetative growth. Growth potential and biomass production of guayule thus were affected by the availability of water during the growth of the plant. The soil structures at the different trial sites were generally of a loam type, well drained, with low nutrients and pH variable between 5-8, which is suitable for guayule growth. Plants grown in less compacted soils such as at Oudtshoorn and Upington, resulted in greater growth potential and biomass production. No apparent trends of nutrient availability in the soil are obvious that explain the different growth responses of guayule in the different areas. In the field mortalities were mainly due to initial poor establishment of transplanted seedlings, but a few successfully established plants were later affected by root rot due to poor soil drainage.

Acknowledgement

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PART 4 GUAYULE LATEX PRODUCTION

CHAPTER VII LATEX PRODUCTION OF GUAYULE CULTIVARS UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Abstract

Latex production of guayule cultivars (AZ-2, AZ-3, N565, 11591) established in trial plots at different areas (Elsenburg, Oudtshoorn, Graaff-Reinet) in South Africa was investigated. Branch samples of one-year old plantings were harvested in April 2002, dipped in 1% ascorbate, sealed in plastic bags and chilled during airfreight to the United States Department of Agriculture (USDA) – Agricultural Research Service (ARS) in Albany CA. Latex extraction and quantification was done and mean latex production and comparisons of latex production for the cultivars in each area were determined. The evaluation of latex production show generally similar results in the different areas. Cultivars generally do not differ significantly from each other in the amount of latex produced in each area. Environmental stress factors on latex production occur in especially Oudtshoorn and Graaff-Reinet where the temperatures are above 25^{O}C and below 10^{O}C. Since guayule is a slow growing shrub, latex accumulation is also slow and takes 4-6 years to reach economic harvesting potential. Production results are therefore preliminary and require further evaluation after each year of growth to present a complete view of guayule latex production over time.

Keywords: guayule, latex production, *Parthenium argentatum*

Introduction

Guayule (*Parthenium argentatum* Gray) is a perennial shrub that originates from the arid to semi-arid regions of the Chihuahua desert, north-central to Mexico and south to south-west of Texas (Lloyd, 1911; Hammond & Polhamus, 1965). It is commercially important due to the ability of the plant to produce latex that can become a natural source of rubber. Furthermore the rubber has non-allergenic properties (Cornish & Siler, 1996), which is of extreme importance considering that many people are allergic (Type IV and Type I) to the current source of natural rubber, *Hevea brasiliensis* (Meuller-Agroviensis, Euphorbiaceae).

Guayule latex is found in most plant parts, i.e. leaves, branches and roots, and is contained in individual cells. The greatest amount of latex is found in the bark, in vascular rays of the phloem and xylem. Lesser amounts are formed in pith, xylem parenchyma and epithelial cells of the resin canals. Very small amounts are present in the leaf parenchyma and in the peduncles (Artschwager, 1943). The habitat of guayule is hot and dry semi-desert areas, where high light intensity is favourable for the production of latex. On soils with high nutrient levels plant growth was found to be improved, though plants grown on low nutrient soils were unaffected (Mitchell *et al.*, 1944). The extreme night cold in these environments also increases latex production and is considered to be a mechanism of survival for the plant (Van Staden *et al.*, 1986). Latex is produced under warm days of $\pm 27^{O}C$ and cold nights of $\pm 7^{O}C$ (Bonner, 1943; Benedict, 1950) and takes four to six years to reach its most economic potential (Hammond & Polhamus, 1965). During seasonal availability of water, active summer growth produce greater biomass, thus increasing production of xylem tissue, but latex production in phloem tissue of mature plants is not enhanced (Lloyd, 1911; Artschwager, 1943).

The initial chewing of the guayule shrub by native Americans and research through the Emergency Rubber Project (Hammond & Polhamus, 1965) has developed procedures focussed on maximum product quantity (latex from bagasse) and quality (rubber from resin). The scarcity of water in arid regions where guayule plantations are envisioned, the cost related to the construction of a processing facility, the infrastructure required for storage of the harvested crop and the preservation of rubber quantity and quality in plants during storage (Taylor & Chubb, 1952) are important considerations that determine the successful commercialization of guayule.

Guayule processing have employed a number of extraction procedures that are based on the three basic concepts of flotation (rubber strands), sequential extraction (first resin then rubber) (Hamerstrand & Montgomery, 1984) and simultaneous extraction (rubber and resin) (Weihe & Nivert, 1980; Wagner & Parma, 1988; Wagner & Schloman, 1991). Further developments by the United States Department of Agriculture – Agricultural Research Services (USDA-ARS) on guayule processing now utilize a standard technique for rapid and effective determining of the latex content of small samples, as used for this analysis (Cornish *et al.*, 1999).

This paper reports on the latex production of guayule cultivars AZ-2, AZ-3, N565 and 11591 established in different areas of South Africa and focus on one-year old plantings at Elsenburg, Oudtshoorn and Graaff-Reinet.

Materials and method

Branch samples of one-year-old guayule plants were harvested in April 2002 from trial plots established at Elsenburg (±34^{0}S; 19^{0}E), Oudtshoorn (±33.5^{0}S; 22.5^{0}E) and Graaff-Reinet (±32.5^{0}S; 24.5^{0}E) in April 2001. Cultivars AZ-2, AZ-3, N565 and 11591 were sampled in all areas except Elsenburg where cultivar 11591 was too small for effective sampling. Plant material was dipped in 1% aqueous ascorbate (to preserve plant tissues) and sealed in plastic bags. These were cooled with ice packs and kept chilled during airfreight to the USDA – ARS Process Biotechnology Research Unit.

Branches were cut (±1cm sections) and immersed in ice-cold, aqueous extraction buffer (0.2% ammonia and 0.1% Na_2SO_3, pH 10) to prevent dehydration of the tissues (1:2, w:v). These were ground twice (1min each, Waring blender 33BL79) and filtered (1 mm steel mesh). Homogenates were thoroughly shaken to suspend the material and three aliquots (1ml each) were pipetted into siliconized microfuge tubes. The tubes were centrifuged in a bucket rotor (2500 x g_n for 15 min at room temperature) to float the latex fraction to the surface. Glacial acetic acid (50 µl) was gently pipetted onto the sample surface so as not to disturb the latex layer and centrifuged again. Fine forceps was used to lift the coagulated latex from the tube, it was rinsed in deionized water, then placed on pre-weighed weighing paper for drying overnight (oven at 37^{0}C), together with three unused weighing papers as blanks. Samples were equilibrated at room temperature for 2h and then weighed to determine the latex yield (Cornish *et al.*, 1999).

Analysis of variance was done on all sample weights to determine mean latex production and compare latex production between cultivars in each area (SAS, 1999).

Results

The probabilities (P) for the factors are greater than 0.05, indicating no significant differences between cultivars (AZ-2, AZ-3, N565) for latex production at Elsenburg (Table 1). Cultivars did not produce significantly different latex products (Figure 1).

Table 1 Analysis table of latex produced by guayule cultivars (AZ-2, AZ-3, N565) at Elsenburg trial plot

FACTOR	CULTIVARS			PROBABILITY (P)	LEAST SIGNIFICANT DIFFERENCE (LSD)
	AZ-2	AZ-3	N565		
LATEX /g STEM FW (mg/g)	14.1500	21.0170	19.7500	0.5679	14.3680
LATEX /g STEM DW (mg/g)	39.0700	60.8300	59.9200	0.5778	49.1930
LATEX /g PLANT FW (mg/g)	5.3670	7.4500	8.3330	0.5439	5.7643
LATEX /g PLANT DW (mg/g)	18.7670	26.3670	28.2830	0.6011	20.9080

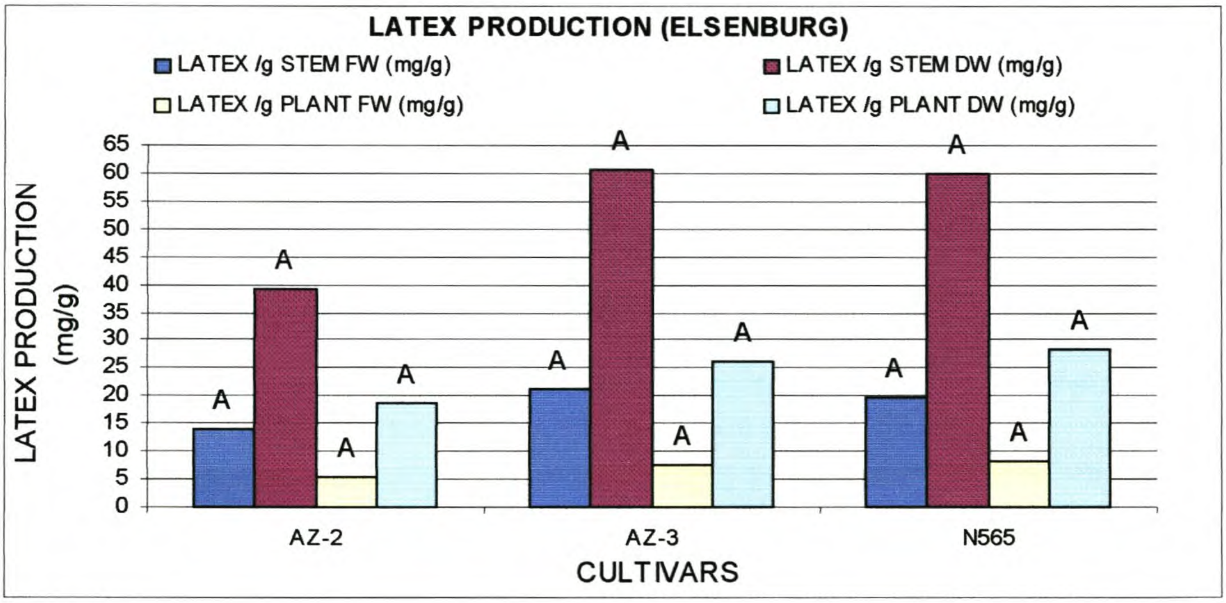

Figure 1 Latex produced from guayule cultivars (AZ-2, AZ-3, N565) at Elsenburg trial plot. Different letters indicate significant differences at p = 0.05 within different parameters.

No significant differences are found at Oudtshoorn between cultivars (AZ-2, AZ-3, N565, 11591) for latex production. The cultivars produced closely similar amounts of latex (Table 2, Figure 2).

Table 2 Analysis table of latex produced by guayule cultivars (AZ-2, AZ-3, N565, 11591) at Oudtshoorn trial plot

FACTOR	CULTIVARS				PROBABILITY (P)	LEAST SIGNIFICANT DIFFERENCE (LSD)
	AZ-2	AZ-3	N565	11591		
LATEX /g STEM FW (mg/g)	16.9000	17.2830	17.0000	29.5500	0.1301	12.6650
LATEX /g STEM DW (mg/g)	39.8000	46.9800	40.5700	63.6200	0.3315	29.6570
LATEX /g PLANT FW (mg/g)	9.0670	9.3330	8.5000	14.8330	0.2333	7.0056
LATEX /g PLANT DW (mg/g)	27.2330	29.2500	24.7830	41.3330	0.3141	19.3040

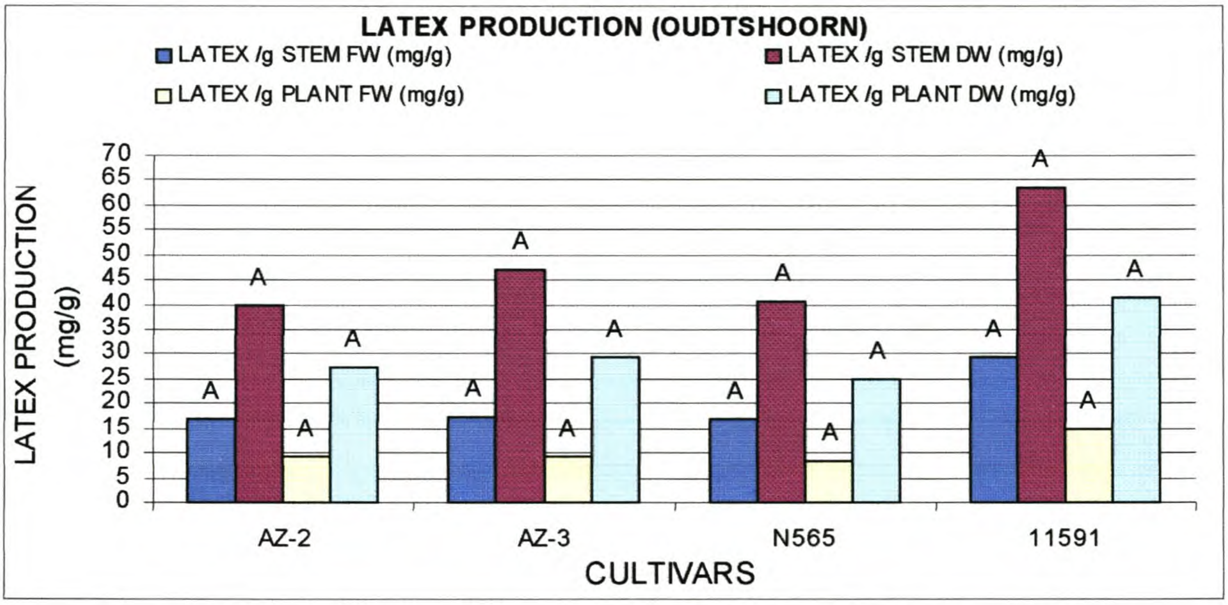

Figure 2 Latex produced from guayule cultivars (AZ-2, AZ-3, N565, 11591) at Oudtshoorn trial plot. Different letters indicate significant differences at p = 0.05 within different parameters.

At Graaff-Reinet significant differences are found between cultivars (AZ-2, AZ-3, N565, 11591) for latex production (Table 3). Cultivar 11591 produced the most latex compared to AZ-2, AZ-3 and N565 (Figure 3).

Table 3 Analysis table of latex produced by guayule cultivars (AZ-2, AZ-3, N565, 11591) at Graaff-Reinet trial plot

FACTOR	CULTIVARS				PROBABILITY (P)	LEAST SIGNIFICANT DIFFERENCE (LSD)
	AZ-2	AZ-3	N565	11591		
LATEX /g STEM FW (mg/g)	12.3830	12.3170	12.0500	28.1000	0.0005	7.7409
LATEX /g STEM DW (mg/g)	27.2670	29.1170	24.4170	56.7170	0.0011	15.6670
LATEX /g PLANT FW (mg/g)	7.7330	7.6330	7.9330	16.5670	0.0305	6.8106
LATEX /g PLANT DW (mg/g)	18.5500	19.8500	18.3500	41.7330	0.0006	11.3730

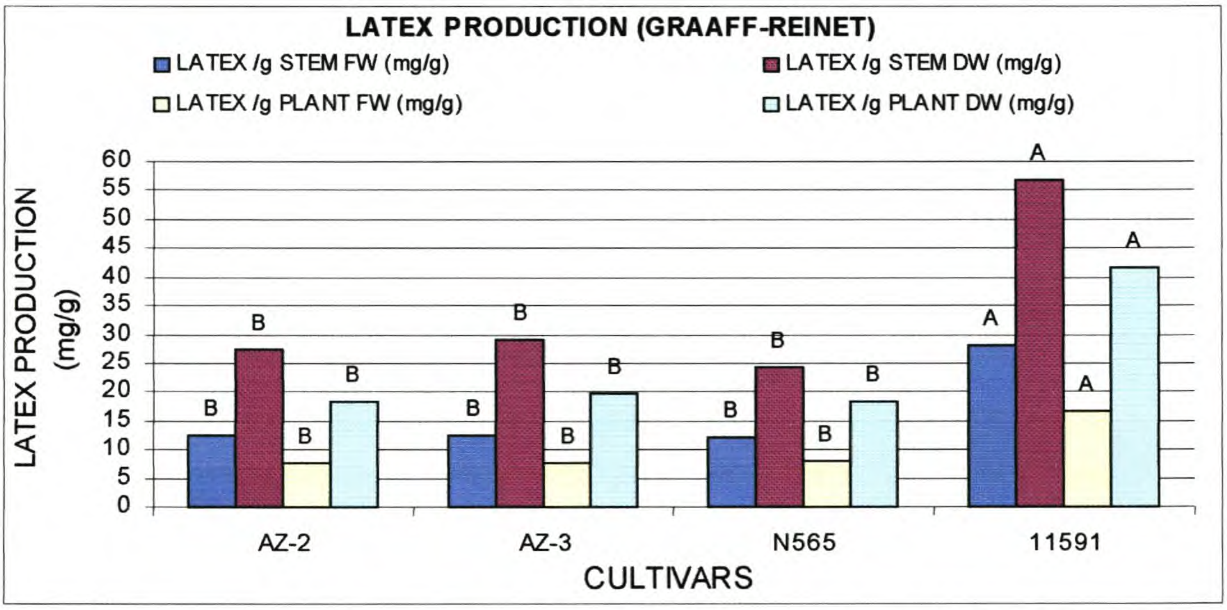

Figure 3 Latex produced from guayule cultivars (AZ-2, AZ-3, N565, 11591) at Graaff-Reinet trial plot. Different letters indicate significant differences at p = 0.05 within different parameters.

Weather data show the highest rainfall for Elsenburg well above 50 mm in May (103.9 mm), July (309 mm), August (212.1 mm), September (120.1 mm) and January (173.3 mm), and average temperature range between 10^0C and 30^0C (Figure 4). Oudtshoorn mostly had rainfall below 15 mm, but had an increase to 40.4 mm in October, with a peak of 73.7 mm in November and a rise to 31.5 mm in February (Figure 5). Average temperature range between 10^0C and 30^0C, with a decrease well below 10^0C in June, July and August. Graaff-Reinet had a rainfall pattern between 50 and 100 mm with peaks in April (92.9 mm, November (76.9 mm), December

(93 mm) January (83.3 mm) and March (67 mm) (Figure 6). Average temperature range between 10^0C and 30^0C, with a minimum below 10^0C from April 2001 to October.

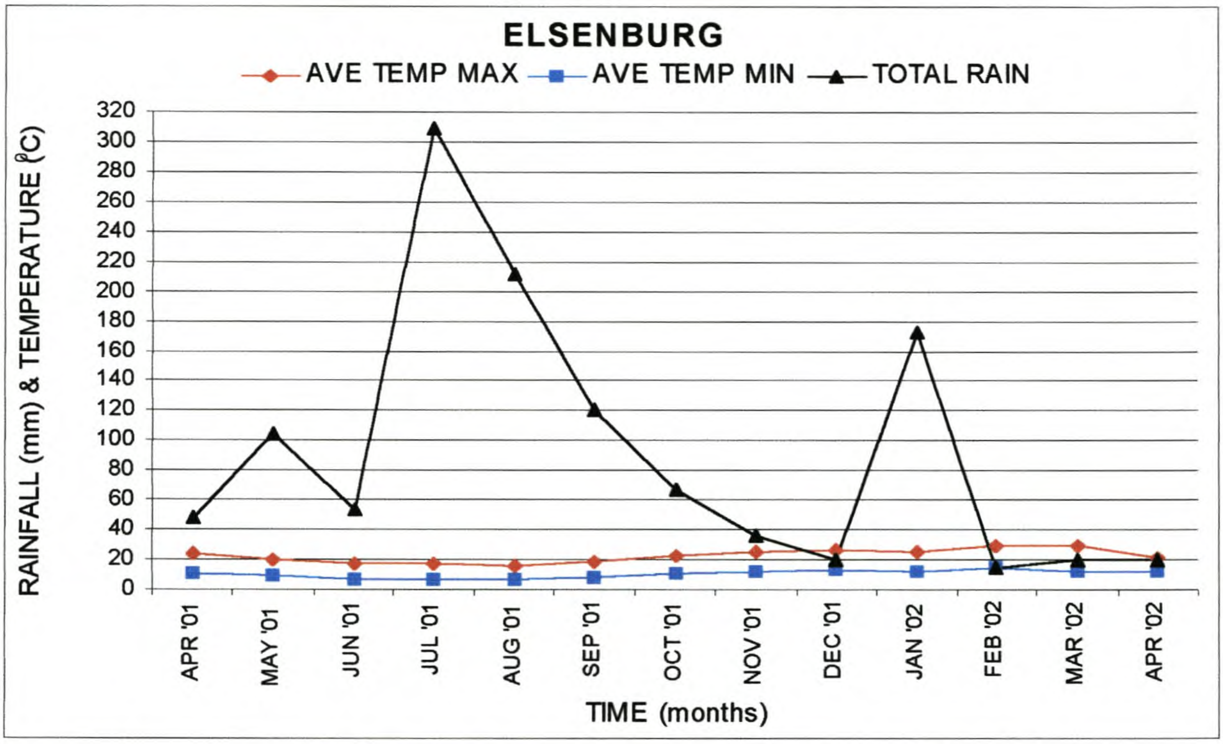

Figure 4 Rainfall pattern and temperature regime of guayule trial plot at Elsenburg.

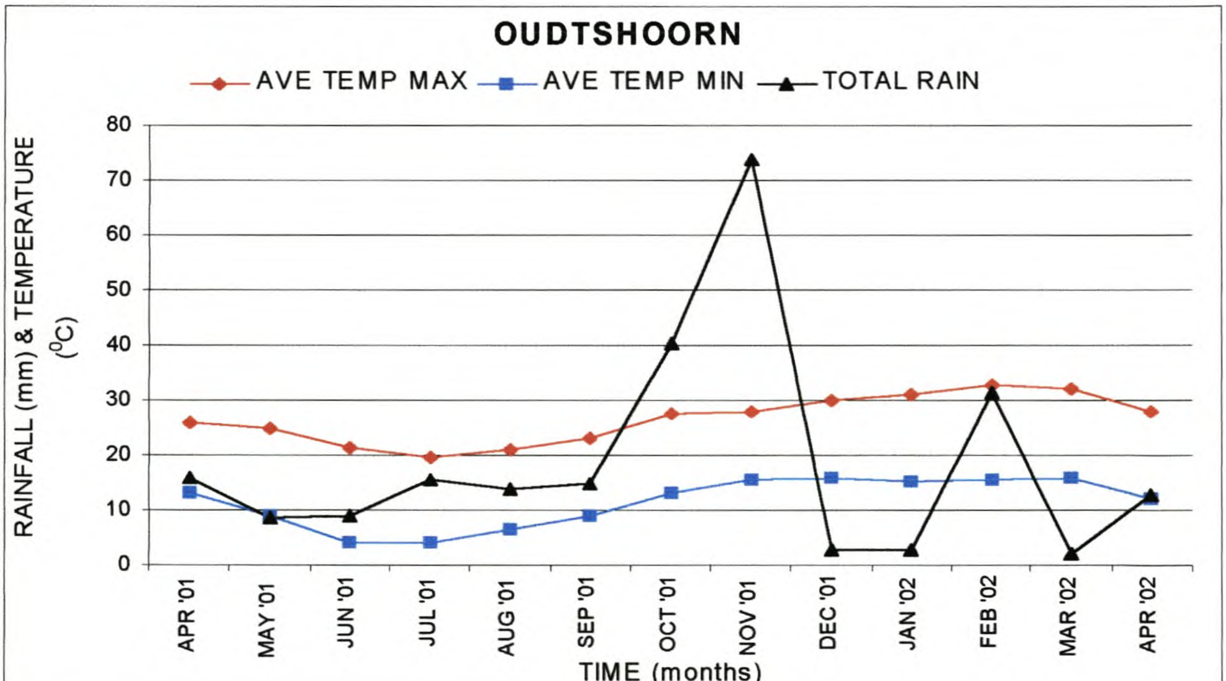

Figure 5 Rainfall pattern and temperature regime of guayule trial plot at Oudtshoorn.

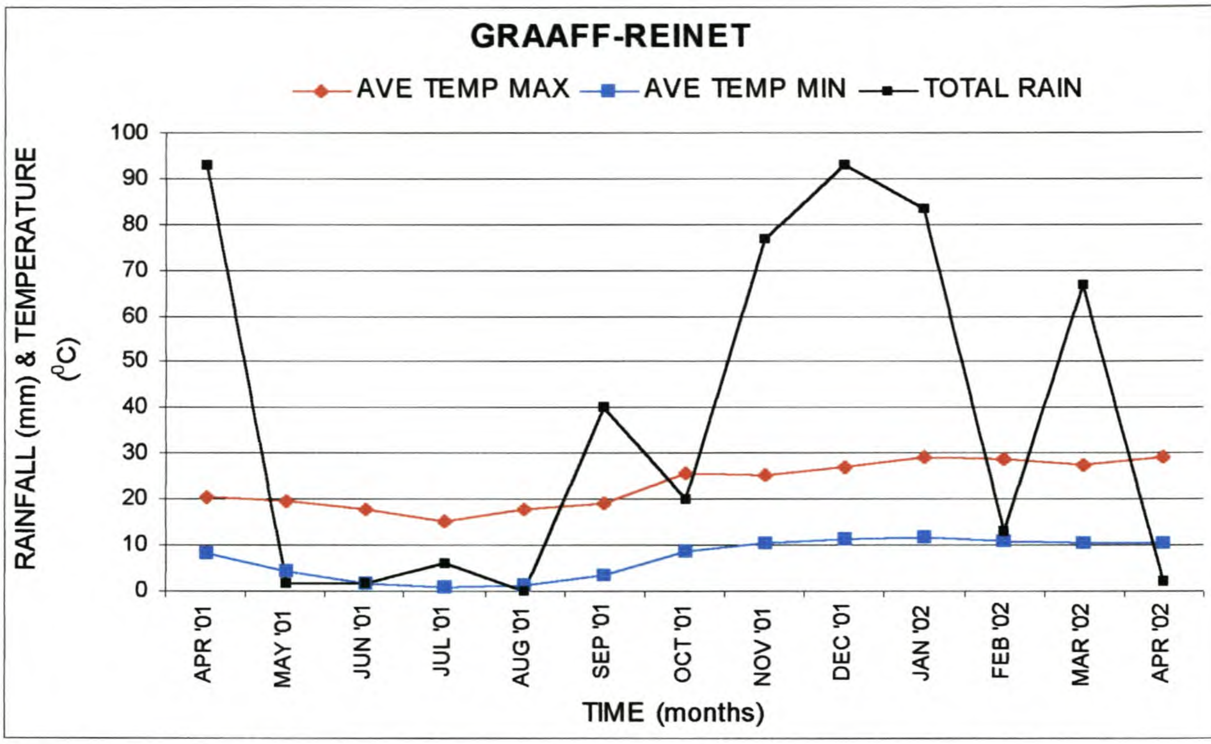

Figure 6 Rainfall pattern and temperature regime of guayule trial plot at Graaff-Reinet.

Discussion

The analysis of guayule latex production after a year of growth show generally similar results in the different areas. Cultivars generally do not differ significantly from each other in terms of the amount of latex produced in each area, with the exception of cultivar 11591. At Elsenburg this cultivar grew too poorly to be sampled, but at Graaff-Reinet it produced the most latex. Cultivar 11591 is a slender shrub that grows weaker than the other bulkier cultivars AZ-2, AZ-3 and N565. Total latex production per square meter of 11591 would thus be less than for the other cultivars.

Guayule latex is found in most plant parts, i.e. leaves, branches and roots, with the greatest amount in the bark, in vascular rays of the phloem and xylem (Artschwager, 1943). Latex production is a mechanism of survival under environmental stress conditions (Van Staden *et al.*, 1986) and is produced under warm days of $\pm 27^{O}C$ and cold nights of $\pm 7^{O}C$ (Bonner, 1943; Benedict, 1950). Environmental stress factors on latex production are well represented in the weather conditions for Oudtshoorn and Graaff-Reinet where the maximum temperatures are above $25^{O}C$ and minimum temperatures below $10^{O}C$.

Since guayule is a slow growing shrub, latex accumulation is also slow and takes 4-6 years to reach economic harvesting potential. In this report, sampling for latex production was done from one-year old plantings. The results are therefore preliminary and require further evaluation after each year of growth to present a complete view of guayule latex production over time.

Acknowledgements

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PART 5 GUAYULE OVERVIEW

CHAPTER VIII ESTABLISHMENT OF GUAYULE (*Parthenium argentatum* GRAY)

Perspectives on study

Guayule (*Parthenium argentatum*) development has a rich history with extensive research of propagation procedures, field evaluations, selective breeding, improvement of latex quantity and quality, product extraction, product analysis, co-product utilization, etc. All these activities were focussed on replacing *Hevea basiliensis* with guayule as a local source of natural rubber, mainly in vehicle tyre manufacturing. In this comparison, guayule never successfully competed with *Hevea* in production potential and also since the envisioned threats of limitations in rubber supply never realized. The whole focus on guayule has changed since the discovery that guayule rubber is non-allergenic to human tissues and blocks transmission of viruses and bacteria. Continued research and development have resulted in improved production potential of selected material, improved analytical latex extraction techniques and varied applications for co-product utilization. The future focus areas of guayule development are the evaluation of seed with selected traits for propagation, field establishment and production potential. Ultimately further development in product manufacturing and market release will be the success indicators of guayule.

Guayule, as a benign plant of the Chihuahuan desert, has experienced years of extensive - though continuously revisited - investigations since 1888. Of all other inquired domestic sources of natural rubber, guayule was found useful in not just producing rubber of comparable quality and strength to *Hevea*, but also distinguished itself in producing rubber with non-allergenic properties and viral impermeability, which makes it extremely valuable in the prevention of disease transmission. Added to this, the whole plant is useful in producing resin, cork, wax, and bagasse. As a crop suited for dryland production, guayule has the potential to be incorporated into agricultural practice in areas that can not be utilized for present crop production due to the lack of sufficient rainfall and poor soil. Since the plant is able to survive extreme temperatures (hot and cold) and does not require intensive soil fertilization, there is great potential to establish farming activities on marginal lands. Technology is currently available on plant physiology, genetics, breeding, propagation, cultivation and agronomy, harvesting and processing to successfully manage guayule as a crop. At the other end of the spectrum, the development of products and markets are also receiving attention from private industry to set up a complete cycle for the commercialization of guayule.

The potential of guayule commercialization in South Africa is vast, especially considering the large areas of suitable land available for production, as well as the economic implications of producing natural rubber locally. Though research expertise on the agricultural practice of guayule is available through collaborative efforts, it needs to be explored and amended for South African environmental conditions and crop management strategies. Research and development of new guayule lines to increase production is a continuous effort to ensure the feasibility of guayule as an alternative crop. From an economic perspective guayule cultivation requires minimal input and low maintenance, but also requires the construction of facilities capable of handling the extraction and processing of products. As with any emerging venture, guayule requires initial drive from private sector, development by industry and a sound commitment by government to be actively involved in the revival of South African agriculture.

Conclusion

Propagation investigations were successful in identifying techniques to germinate guayule seed and promote rooting of cuttings with specialized treatment solutions. Though results indicate an improvement with treatment, more effective means of seed germination and rooting procedures are required. Field establishment of guayule under South African environmental conditions has identified suitable areas and indicated cultivar performances in these areas. Evaluation of the latex production of field plantings has demonstrated the potential of guayule in these areas. These research elements will be drawn on again for the improvement of current results and further evaluation as new cultivars are developed. There is a need for economic studies to determine the feasibility of guayule farming in South Africa. Furthermore, large-scale operation requires an extraction facility for the processing of the raw products. Input costs at this level require investment from private industries and government.

Successes of guayule breeding, propagation, establishment, latex extraction and product development have been distinct efforts to uncover the potential of guayule as an agricultural crop. Currently the path to guayule development is paved with a network of research activities that is strengthened through cooperation between research institutions and private sector companies that bridge the gap between academic research and market exhibition. The future challenge is to maintain this momentum in a coherent drive to guayule commercialization.