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Evaluating New Guayule Varieties for Low-Allergenic Rubber Production

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Foreword

Guayule, a crop suited to semi-arid areas, produces latex that is low-allergenic and is thus vital to people who may suffer life-threatening allergies caused by tropical Hevea rubber. There is an increasing demand for high end rubber products for medical use such as rubber gloves and catheters as well as condoms. The availability of Hevea rubber is decreasing due to reduced labour and competition from other crops. Synthetic rubber, while being non-allergenic, does not possess the resistance to viral transmissivity of guayule rubber, and its price is increasing due to increased petroleum prices. Australia has large areas of land with soils and climate suitable to guayule production. Yulex Corporation (U.S. based) has expressed interest in setting up a pilot plant in Australia to process the crop.

The report describes three major areas of research: evaluation of USDA germplasm, seed dormancy and direct seeding. New U.S.D.A. lines generally produced higher biomass, rubber yields and resin yields from stem and branches compared with old lines. AZ-1 and AZ-2 produced consistently significantly higher rubber yields than the old lines at both Gatton and Chinchilla. AZ-5 produced the highest rubber yield of 966 kg/ha at Gatton in the third year (an increase of 43% over the old lines); however, plant type was variable indicating potential for further selection. Increased rubber and resin yields from these new lines ensure that guayule is economically more competitive than in the past.

The seed dormancy and direct seeding research are important in reducing the establishment costs (transplants) of the crop. The seed coat serves as a mechanical barrier to the emerging radicle as well as a source of germination inhibitors. Further, this research has shown that the light requirement can be totally replaced by exogenous gibberellins but the concentration varies with the level of dormancy. Direct seeding research demonstrated that the crop can be successfully established using a fine seedbed, shallow precision planting, post-plant irrigation and effective weed control. This results in much reduced establishment costs.

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Peter O'Brien
Managing Director
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Contents

Executive Summary

Over 2000 plant species produce rubber. However, only two plant species produce high molecular weight rubber of commercial interest. These two plant species are the tropical tree, *Hevea brasiliensis* and the semiarid shrub, guayule (*Parthenium argentatum* Gray). At present, *Hevea brasiliensis* is the sole source of natural rubber. However, continuity of supply of rubber from *Hevea* is insecure because of risk of crop failure and diminishing area of production. Moreover, *Hevea* causes life-threatening Type I latex allergy which is triggered by the presence of protein in the latex. Latex allergy caused by *Hevea* latex has become a major health hazard since the 1980s with the increased use of latex products (medical gloves and condoms) to control the outbreak of deadly diseases like Acquired Immune Deficiency Syndrome (AIDS). Of the three types of allergy, Type I immediate hypersensitivity or IgE mediated anaphylactic reaction is life threatening and is caused by proteins in the *Hevea* latex. It was reported that almost 10% of the population in the USA are allergic to *Hevea* latex.

Guayule (*Parthenium argentatum* Gray) is a semi-arid plant that produces high quality natural rubber and low-allergenic latex with the potential to become commercial crop. Natural rubber possesses high performance properties that cannot be achieved by synthetic forms. These include resilience, elasticity, abrasion resistance, efficient heat dispersion, impact resistance, and malleability at cold temperatures. Due to its superior quality, natural rubber is an essential raw material for many products. It is often blended with synthetic rubber for various products but many are 100% natural rubber (e.g. medical gloves and condoms). Although synthetic rubber is non-allergenic, it is becoming more expensive due to increased petroleum prices. Australia imported $ 1,045 million worth of rubber tyres, tubes and related products during the financial year 2001-2002 that accounted for 0.9% of total imports.

Previous intermittent attempts to commercialise guayule in the USA and Australia highlighted the need for further research to increase rubber yields and/or decrease production cost. Latest research has resulted in considerable progress in the release of high yielding lines. New releases produced significantly higher rubber and resin yields compared to existing lines in environmental conditions in the USA, however, these new lines have not been tested in the Australian environment. Therefore, the broad objective of this research was to investigate the commercial potential of guayule in Australia. Experiments were conducted with three specific objectives, firstly, to evaluate the performance of new USDA guayule germplasm under different environmental conditions within two potentially suitable areas in southeast Queensland. Secondly, to gain further understanding of the mechanisms of seed dormancy to facilitate direct seeding. The third objective was to investigate the potential of direct field seeding as an alternative to expensive transplanting.

Evaluation of USDA germplasm

Results of germplasm evaluation trials verified that both environments Chinchilla (300 km northwest from Brisbane) and Gatton (80 km west of Brisbane) were suitable for guayule production. Overall performance of plant growth, dry matter, rubber and resin yields (stem and branches) of six new guayule lines at two sites in Queensland was generally better than the old lines (N565 and 11591). Of all the lines, AZ-1 and AZ-2 were the best for both environmental conditions. They produced early vigorous growth, increased dry matter, increased rubber and resin yields. They also can be harvested in shorter duration (two years) in good soils and environmental conditions. Of these two lines, AZ-2 was preferred over AZ-1 due to its comparatively high uniformity.

AZ-1 and AZ-2 produced rubber yields of 567 kg/ha and 611 kg/ha respectively in the second year (17 months) at Gatton from stem and branch that was 48% to 61% higher than the old lines. These two lines (AZ-1 and AZ-2) produced rubber yield of 717 kg/ha and 787 kg/ha respectively from stem and branch in the third year (33 months) at Chinchilla. Thus the yield increase over the old lines was between 86% and 107%. No ratoon studies were performed.

AZ-5 produced the highest rubber yields from stem and branch at both the second and third year at Gatton, 565 kg/ha and 966 kg/ha respectively. This is over 43% increase in yield over the old lines. However, the uniformity of AZ-5 was lower than that of AZ-1 and AZ-2 indicating that further selection is possible. Also it did not perform better at Chinchilla.

The yield improvement in both rubber and resin was due mainly to improved biomass and was evident for most of the new lines. Rubber yields approaching 1000 kg/ha were achieved under well irrigated, fertile conditions indicating the improved returns for these new lines. Higher yields may be achieved under higher plant populations (this study used a plant population of 19,000 plants/ha but 27,500 plants/ha has been used in the U.S.). No studies were performed on ratoon crops although measurements were made on root yields at Chinchilla; the recommendation in the U.S. has been to harvest at two years and ratoon annually each spring with a final harvest in year five when the roots as well as tops are harvested.

At Gatton, plant death was noted in some plots after the soil became waterlogged after summer rain. This was ascribed to *Rhizoctonia* sp. and confirms the finding of previous researchers who found that slow draining cracking clay soils were prone to disease under these conditions.

Seed dormancy
Seed germination experiments revealed that dormancy or germination in guayule was regulated by the balance of growth promoters and inhibitors. Dormancy and germination of guayule seed depend upon the corresponding shift in the threshold levels of growth promoters and inhibitors. Endogenous or exogenous growth regulators can shift the balance to inhibit or induce germination. Further, the level of dormancy or rate and extent of germination depends upon the degree of shift from the threshold levels.

High levels of dormancy with freshly harvested seed are due to high levels of inhibitor (abscissic acid or ABA and/or phenolic compounds) and/or low levels of promoter (gibberellic acid or GA). Parent environmental conditions during seed development determine the levels of growth regulators (GA and ABA) and hence the dormancy.

The seed coat also contains inhibitors that affect the balance between promoter and inhibitor. Therefore, gibberellins are required to overcome germination constraints imposed by the seed coat and ABA-related embryo dormancy. The seed coat also acts as mechanical barrier to the emerging radicle.

Light requirement or response to gibberellin in germination of guayule is mediated through phytochrome, a plant hormone. Therefore, light and its quality are also important to activate phytochrome that induces the synthesis of gibberellins to promote germination. Further, the light requirement in germination can be totally replaced with exogenous gibberellin acid. However, the required amount or concentration varies according to the level of dormancy.

Direct seeding
Direct seeding trials indicated that establishment by direct seeding is possible. However, high quality seed, fine textured uniform seedbeds, shallow precision planting, adequate soil moisture throughout the first three weeks and efficient weed control are required to achieve good establishment by direct seeding. Direct seeding is a challenge due to small seed size (1 mg) and the need to have a shallow depth. Soil moisture must be sustained long enough to allow germination and establishment.

Osmopriming with polyethylene glycol was very effective in increasing germination, emergence and establishment. It also improved seedling growth at the initial stage.

Implications and recommendations

This research has identified suitable lines for guayule production in south-east Queensland. These lines have greatly improved rubber and resin yields due to improved biomass compared with old lines. Improvement in both these attributes will increase the economic viability of the crop. Australia has large areas of land with soils and climate suitable to guayule production. Yulex Corporation (U.S. based) has recently expressed interest in setting up a pilot plant in Australia to process the crop. There is thus considerable opportunity for import replacement from guayule production; however, the major use for guayule rubber may come from high end medical uses which need a low-allergenic, high quality source.

Further increases in yield are possible through higher plant population, harvest after two years and ratoon crops. Further research is necessary to characterise performance under these conditions. It was evident from the variability of growth of some varieties (such as AZ-5) that further selection could be undertaken with potential for increased yields.

A note of caution is needed regarding soil type. Guayule appears to perform well on slow draining cracking clay soils until waterlogging occurs. This can result in rapid plant death and may necessitate planting of the crop on well drained soils such as sandy loams. Further research is being conducted at the University of Queensland to investigate this aspect.

Greater understanding of guayule seed dormancy has been achieved especially in relation to the effect of the embryo (germination inhibitor) and seed coat which is both a mechanical barrier to radicle emergence and a location for germination inhibitor. This understanding is very useful in treating seed to improve germination and thus establishment, both by direct seeding and transplanting.

Direct seeding trials demonstrated that the crop can be established by this method albeit under well controlled conditions. This can greatly reduce establishment costs and increase the attractiveness of the crop commercially. Where the crop is grown under dryland conditions, further research is necessary to identify sowing times and cultural practices such as mulching which would increase establishment rates.

A further issue that was recognised in undertaking the present project was the need to develop more efficient methods of harvesting and processing seed. Seed is produced over an extended period during the summer and shatters readily once it is physiologically mature. Seed size becomes an issue for cleaning and processing. Research on these areas is being undertaken at the University of Queensland and will facilitate the production of clean seed of high quality for direct seeding.

1. Introduction

1.1 Importance of rubber

Rubber (cis -1,4-polyisoprene) is one of the most important raw materials in the world and is used for the manufacture of more than 40,000 products, including over 400 medical devices (Mooibroek and Cornish 2000; Cornish 2001). It takes two forms, natural and synthetic. Synthetic rubber derived from petroleum accounts for 60% of total rubber consumed annually in the world. The other 40% (6 million tons) is a natural product from plants (Auchter *et al*. 2000). Natural rubber possesses high performance properties that cannot be achieved by synthetic forms. These include resilience, elasticity, abrasion resistance, efficient heat dispersion, impact resistance, and malleability at cold temperatures (Mooibroek and Cornish 2000; Cornish 2001). Due to its superior quality, natural rubber is an essential raw material for many products. It is often blended with synthetic rubber for various products but many are 100% natural rubber (e.g. medical gloves and condoms).

Australia imported \$ 1,045 million worth of rubber tyres, tubes and related products during the financial year 2001-2002 that accounted for 0.9% of total imports (Trewin 2003).

1.2 Rubber producing plants and latex allergy

Over 2000 plant species produce rubber. However, only two plant species produce high molecular weight rubber of commercial interest (Thompson and Ray 1989) with an average molecular weight of ca 3-7 x 10^5 (Backhaus 1985). These two plant species are the tropical tree, *Hevea brasiliensis* and the semiarid shrub, guayule (*Parthenium argentatum* Gray). At present, *Hevea brasiliensis* is the sole source of natural rubber. However, continuity of supply of rubber from *Hevea* is insecure because of risk of crop failure and diminishing area of production (Mooibroek and Cornish 2000).

Hevea causes life-threatening Type I latex allergy which is triggered by the presence of protein in the latex (Siler and Cornish 1994). Latex allergy caused by *Hevea* latex has become a major health hazard since the 1980s with the increased use of latex products (medical gloves and condoms) to control the outbreak of deadly diseases like Acquired Immune Deficiency Syndrome (AIDS). Of the three types of allergy, Type I immediate hypersensitivity or IgE mediated anaphylactic reaction is life threatening and is caused by proteins in the *Hevea* latex. It was reported that almost 10% of the population in the USA are allergic to *Hevea* latex (Anon 2000).

1.3 Guayule as a potential alternative

Ever increasing demand for natural rubber and the life-threatening allergy to Hevea latex has created a challenge to develop an alternative rubber crop. The Mexican rubber plant, guayule, from which commercial rubber was extracted during the early 1900s, has been envisioned as the best alternative for this purpose.

Commercialisation of this crop has been attempted three times during the last century. The latest major attempt was commenced during the 1970s when synthetic rubber prices increased due to increases in crude oil prices (Henderson 1983; Ray 1993). These attempts have made considerable progress in improving the commercial potential of guayule. It also has been tested and proven to produce high quality latex suitable for manufacture of hypoallergenic products (Siler and Cornish 1994; Cornish and Siler 1996).

1.4 Potential to grow guayule in Australia

Guayule requires grows well in semiarid climate with well-drained soils with medium to fine textured subsoils, slightly acid to alkaline reaction, and with low salt content. Australia has a large area of semi-arid land with soils and environmental conditions potentially suitable for guayule. An area extending from Wagga in New South Wales to Charters Towers in Queensland in eastern Australia within the 500-700 mm rainfall belt has the most favourable conditions. Within this region, the main soils that appear to have the required characteristics are red earths and well-drained duplex and clay soils. Based on climatic and soil factors, it was estimated that the gross area of potentially suitable soils was approximately 6 million hectares (Stewart and Lucas 1986).

1.5 Limitation in commercialisation

Guayule needs to be competitive with *Hevea* to become a commercial source of natural rubber. Low annual rubber yields (600 - 900 kg ha^{-1}) and high establishment costs (about A$1800 in Australia (Jackwitz, 2003) and US$ 1600 in the U.S. (Foster *et al*. 2004)) are two of the major limitations to commercialisation of guayule (Estiliai 1991; Foster and Coffelt, 2004). Research programs including the development of high yielding lines with faster growth have been carried out to address these important issues.

Commercial utilisation of guayule co-products also needs to be explored. The main co-products that are produced in large quantities in processing of guayule are low molecular weight rubber, resins and bagasse. Utilization of these products is crucial to improve the commercial potential of guayule (Wright *et al*. 1991).

1.6 Objectives of this research

The aim of this research was to develop a guayule production system for low-allergenic rubber in Australia. The specific objectives were to:

a) evaluate the performance of new USDA guayule germplasm under different environmental conditions within two potentially suitable areas in southeast Queensland, Australia.
b) fill the knowledge gaps in mechanisms of seed dormancy to understanding why seed treatments are effective.
c) develop a direct field seeding method using appropriate machinery, techniques and seed treatment method, and thus reduce the establishment costs of transplanting.

2. Literature review

2.1 Introduction

Development of guayule production system for commercial rubber requires a thorough understanding of the crop's agronomy and climatic and soil requirements. Dormancy mechanisms, seed treatment and enhancement methods developed for other crops is also very important in acquiring knowledge that could helpful in planning experiments on investigation on seed dormancy and development of a direct seeding method for guayule. Therefore, this chapter covers literature in two main areas under several sub-topics. Main part of this chapter reviews current literature available on guayule with the objective to present this information and indicate gaps in the knowledge base. Under this Australia's agronomic potential to produce commercial rubber from guayule has also been reviewed. The second main part covers findings on seed dormancy mechanisms and seed treatments methods used to enhance field establishment of range of crops including guayule with objectives to indicate gaps in guayule in relation to seed dormancy and establishment and to use available knowledge developed with other crops to improve commercial potential of guayule.

2.2 History of the development of guayule

2.2.1 Mexico and USA

Guayule commercialisation began with the harvesting of wild stands in Mexico. The first commercial utilisation of guayule rubber dates back to 1888 (Hammond and Polhamus 1965; Estilai and Ray 1991). In 1910, Mexican rubber production (from wild stands) reached a high of 10,000 t from about 20 mills (Bonner 1991; Ray 1993; Cornish and Siler 1996) and accounted for 10% of the world's natural rubber requirements (Anon. 1977). Subsequently, production in Mexico declined due to revolutionary disturbances (Bonner 1991).

Thereafter, three attempts have been made in the United States to grow guayule as a cultivated crop (Huang 1991). The Continental Mexican Rubber Company moved its headquarters and processing plant to Salinas, California, due to the revolutionary disturbances in Mexico, and became the Intercontinental Rubber Company (Bonner 1991). The first attempt to grow guayule as a cultivated crop was initiated by the Intercontinental Mexican Rubber Company which planted 3000 ha (Huang 19991; Bonner 1991). During the late 1920s the Intercontinental Mexican Rubber Company started producing guayule rubber from their plantations at the rate of about 1400 tons per year. Production ceased at the beginning of the Great Depression in 1929 (Ray 1993; Bonner 1991; Cornish and Siler 1996).

The second attempt to commercialise guayule was initiated during the Second World War. During this period, the United States was cut off from the supply of natural rubber derived from *Hevea*. This led to the inauguration of the Emergency Rubber Project (ERP) to supply the strategic raw material. The project employed 1,000 scientists and technicians, 9,000 labourers and had 13,000 ha of guayule under cultivation in three states. More than 1,400 t of rubber was produced during the period (Hammond and Polhamus 1965). The ERP was terminated at the end of the war and the remaining guayule planting (85%) were destroyed due to the increased availability of Hevea rubber, an over-optimistic view of the utility of synthetic rubber, and international political pressure (Hammond and Polhamus 1965; Cornish and Siler 1996).

The third and current attempt started during the 1970s with the onset of increased oil prices. High oil prices led to increased costs of synthetic rubber and renewed interest in guayule as an alternative source of natural rubber. The current continuing efforts have made considerable progress in improving guayule's commercial potential through plant breeding, agronomy (Cornish and Siler 1996) and processing (Wagner and Schloman 1991).

Further, recent research has shown that guayule latex is hypoallergenic, and therefore can be used to produce high-value latex medical products especially for personnel sensitive to Hevea latex (Siler and Cornish 1994). The Agricultural Research Service of the United States Department of Agriculture (USDA) has developed a procedure to produce hypoallergenic latex from guayule (Cornish 1996). This procedure is patented and licensed to the Yulex Corporation in the USA for the production of high value latex products (Wood 1997). Yulex Corporation now has started production of guayule for the manufacture of high value medical products.

2.2.2 Australia

Australia has made two main attempts to grow guayule during the last century. The first attempt which had both research and commercial focus, was reported during World War II. The fall of Malaya to the Japanese in December 1941 produced a drastic change in the world rubber position, and early steps were taken in Australia to investigate alternative sources of natural rubber. Owing to the critical stage of the war, the earliest trials were conducted on an extensive scale near Adelaide, South Australia, under the joint supervision of the Council for Scientific and Industrial Research (CSIR now CSIRO) and the Waite Agricultural Research Institute (Crocker and Trumble 1945).

Rapid expansion of field planting failed owing to difficulties with seed germination and seedling establishment during 1942-43. With improvements in the war position in 1943, Hevea rubber became more readily available thus re-orientering the project, which became purely investigational. During this period, factors governing seed germination, nursery production of seedlings, and seedling establishment were determined (Crocker and Trumble 1945).

An agronomic analysis carried out in 1979 indicated that guayule appeared suited to much of the eastern cereal belt of Australia (Nix 1986). The second main attempt focused on field experiments in New South Wales in the early 1980s to evaluate growth potential and agronomic requirements. Through the co-operation of the USDA and various United States agencies, 22 small trial plots were established at sites extending in a broad belt from the far north to the south of New South Wales, principally in the western wheat belt (Milthorpe 1984).

During this period, various factors affecting field production of guayule were investigated. Low soil pH (5 or less) and water logging that increased incidence of root diseases were detrimental; establishment and weed control needed special attention (Milthorpe 1984).

Later, parallel to the New South Wales investigations, the CSIRO Division of Tropical Crops and Pastures carried out an experiment at Kingaroy in Queensland. This trial reported up to 13% rubber content, higher than that found in New South Wales and USA. However, plants were affected by root disease (Fusarium spp.) resulting in low leaf area and low biomass yield (a maximum of 9.5 t/ha in 36 months) (Ferraris 1993).

2.3 Botany of guayule

2.3.1 Morphology

Guayule, Parthenium argentatum Gray, is a small silver gray, woody perennial shrub belonging to the tribe Heliantheae of the family Asteraceae (West et al. 1991). Out of the 16 species in the genus, guayule is the only species that produces high molecular weight rubber of commercial interest (Thompson and Ray 1989) with an average molecular weight of ca 3-7 x 10^5 (Backhaus 1985). Natural stands of guayule show considerable genetic variability for rubber content (5 to 17%) but average about 10% (Goss 1991). Unlike the rubber in Hevea, guayule rubber is not contained in ducts but is suspended in thin wall cells found mainly in the bark (Wanger and Schloman 1991).

Guayule is a long-lived plant that may survive 30 to 40 years under the harsh desert conditions of its native habitat (Siddiqui and Lucktov 1981). It is a multi-branched bushy plant (Figure 2.1) which produces inflorescences on long peduncles on the upper layer from spring to autumn. In its natural habitat, the height of mature guayule plants is about 0.5 to 0.6 m and rarely exceeds 1 m (Lloyd 1911). Under cultivation, guayule shrubs can grow to about 1.3 m in height and 1.4 m in width (Thompson and Ray 1989).

Root System

The root system consists of the taproot, which may lose prominence and give way eventually to an intricate system of dense fibrous laterals and their branches (Artschwager 1943; Muller 1946). The depth of penetration and subsequent suppression of the taproot is determined by the depth of penetrable soil. Roots of plants in wild stands investigated in Texas rarely penetrated the soil beyond 60 cm. The greatest concentration of fibrous roots is in the upper 15 cm of soil (Hammond and Polhamus 1965).

Stems

The first inflorescence is usually formed during the first year of growth and it terminates the monopodial growth of the plant. This stimulates the active growth of two or three of the uppermost buds. Each of these ends its growth by the formation of an inflorescence. This system of branching continues resulting usually in a symmetrical and closely branched shrub (Figure 2.1) (Hammond and Polhamus 1965).

Flower and fruit

Guayule flowering occurs in periods of active growth when adequate water is available. A minimum of 9.5 hours of day length is required to stimulate flowering (Backhaus et al. 1989). Flowering is most abundant in spring but also occurs through summer and autumn if sufficient water is available. Guayule is both wind and insect pollinated. Seeds are very small and are produced at a prolific rate (Hammond and Polhamus 1965).

The inflorescence is a compound, one-sided cyme and the flowers are borne in heads on a common receptacle. The head contains five fertile ray-florets, each with two attached subjacent sterile disk-florets (Figure 2.2). The ray-florets are unisexual with no visible remnants of stamens. The disk-florets contain an abortive pistil in addition to the fertile stamens and, with the exception of the outer row, are attached to each other at the base, and fall from the flower head as a unit. The achene complex, when it is shed, consists of the achene to which are fused, at its base, the two subjacent sterile-florets and a subtending bract, together with persistent ligule and the withered two-lobed stigma (Artschwager 1943; Hammond and Polhamus 1965). The mature achene contains an embryo invested by two seed coats, an outer one that is thin, white, and soft and an inner one that is thin, white and tough (Benedict 1946).

Figure 2.1 A twenty months old guayule plant without inflorescences (at the beginning of winter)

Leaves

The narrow leaves of guayule are grown in clusters and covered with drought-protecting white wax. Both upper and lower surfaces are densely covered with T-shaped, nearly sessile trichomes laid parallel to the leaf axis, thus producing the light green-grey sheen characteristic of the plant.

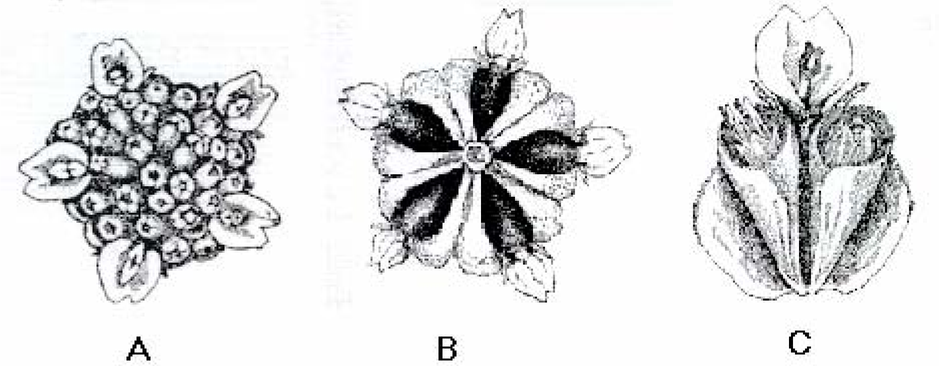


Figure 2.2 (A), Surface view of flower head, (B) Rear view of a mature flower head with bracts removed. (C), Enlarged view of a ray floret with its 2 adjacent disk-florets (Artschwagwer 1943).

Morphological variation

Mehta et al. (1979) used trichome morphology with leaf shape and rubber content to distinguish morphological and biochemical variations among plants in native habitats of guayule. They suggest that varying degrees of introgression by Parthenium incanum H.B.K. (mariola), a plant that grows in close association with guayule in its native habitat may be responsible for many of the morphological and biochemical differences among native populations. They categorised guayule plants into three groups as follows:

Group I

Plants in this group are generally the smallest of the three groups and have the highest percentage of rubber, averaging 17%. Leaves are oblanceolate in shape and commonly have one to two teeth, very rarely with three or more teeth (Figure 2.3AI). Leaf length ranges from 2.5 to 4.3 cm and width 0.4 to 1.0 cm. Both upper and lower epidermal leaf surfaces of leaves are covered with two types of trichomes. More common are the T-shaped trichomes with an unicellular cap cell and a 1 to 2 celled stalk. The stalk is attached centrally to the cap cell. Most of the cap cells are blunt at both ends.

The inflorescences are compound with a group of heads elevated on a leafless inflorescence axis ranging in length from 9 to 22 cm (Figure 2.3BI). Usually there is only one branch, near the tip, and the branch extends beyond the main inflorescence axis.

Group II

Average rubber content in plant of this group is 10 percent. The leaves are oblanceolate to narrow elliptic in shape (Figure 2.3AII). The length of a leaf ranges from 1.4 to 5.7 cm and width from 0.4 to 1.4 cm. Leaves have two to four teeth and surfaces are predominantly covered with T-shaped trichomes. Trichome stalks are attached acentrically to the cap cell. The short end of the cap cell is blunt and the long end is straight or slightly hooked and pointed.

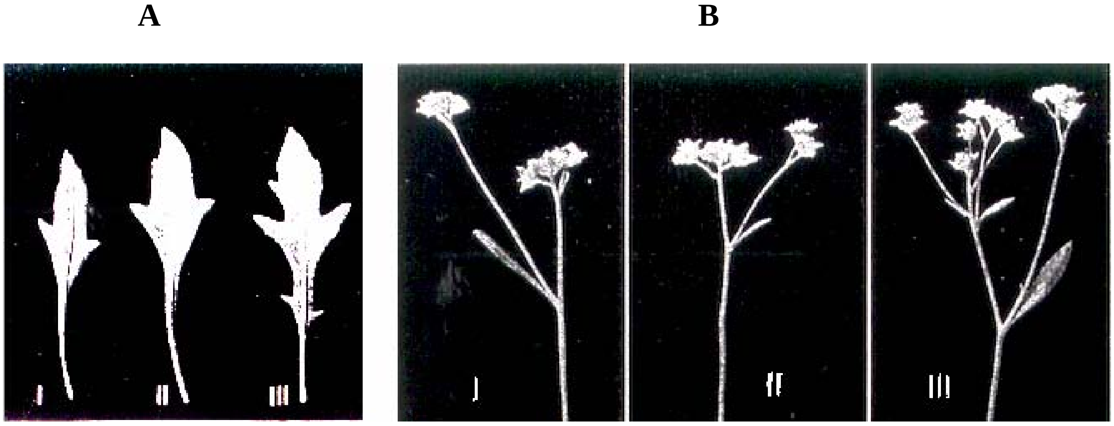


Figure 2.3 (A) Leaves of group I (I), group II (II), and group III (III), (B) Inflorescences of group I (I), group II (II), and group III (III) (Mehta *et al.* 1979)

The inflorescence axis ranges from 9-20 cm in length and branches only once near the tip with the branch extending beyond the main axis (Figure 2.3BII). The peduncles are short and heads are grouped compactly.

Group III

Morphologically this group of plants is more distinct than the previous group. The leaves are generally ovate in shape; the length ranges from 1.8 to 6.0 cm and width ranges 0.6 to 1.3 cm (Figure 2.3AIII). The leaf margins generally have four to eight teeth; very rarely they possess less than three teeth. The stalks of T-trichomes are longer, 1 to 3 celled and are attached acentrically to the cap cell. The cap cells are considerably longer than in the previous two groups, averaging 238.2 µm. The short end of the cap cell is straight and blunt, whereas the long end is wavy and pointed.

The inflorescence axis is usually shorter than the other groups, ranging from 5 to 15 cm and has three to four branches (Figure 2.3BIII). All the branches are at about the same height as the main inflorescence axis and the peduncles are 3-7 mm long. The rubber concentration in this group of plants is low, ranging from 2 to 9% with an average of 6%.

2.3.2 Genetics and reproduction

Varied chromosome numbers

Natural populations of guayule contain plants with different levels of ploidy (Thompson and Ray 1989). In wild stands, guayule is reported to have polyploid series consisting of diploids ($2n = 2x = 36$), triploids ($2n = 3x = 54$) and tetraploids ($2n = 4x = 72$) (Bergner 1946). In addition to these ploidy levels, Kuruvadi *et al*. (1997) found the presence of pentaploids ($2n = 5x = 90$) and aneuploids in natural stands. The natural distribution of these ploidy levels was observed to be 2.1% diploids, 12.8% triploids, 78.5% tetraploids, 3.1% pentaploids and 3.6 aneuploids (Kuruvadi *et al.* 1997). The ploidy level has a profound effect on the morphological, anatomical, physiological and genetic characteristics, and reproductive behaviour of guayule (Kuruvadi *et al.* 1997).

Mode of reproduction and self-incompatibility

The mode of reproduction in diploid guayule plants with $2n=36$ chromosomes is sexual. These diploids are outcrosses because of the inability of their pollen to grow on the surface of the stigma of their own flowers and to fertilise the eggs (Estilai and Ray 1991). The sporophytic system of incompatibility was found in guayule (Thompson and Ray 1989; Estilai and Ray 1991). The presence of the same R allele in the pollen and stigma will inhibit germination of the pollen grain or prevent the pollen tube from penetrating the stigmatic surface. The drawback of self-incompatibility in guayule has been the failure to produce homozygous genotypes (Estilai and Ray 1991). Diploids, in general, are associated with slow growth, low vigour, short stature and small top spread with resultant low biomass and rubber yield. However, they are useful as female parents in hybridisation with plants of different ploidy levels, as well as for inter-specific and intra-specific crosses (Kuruvadi *et al*. 1997).

Naturally occurring triploid and tetraploid guayule plants reproduce mainly by apomixis, but these polyploids produce residual sexual seed by normal meiosis followed by fertilisation. Thus, this mode of reproduction, known as facultative apomixis, results in both uniform apomicts and diverse sexual progenies (Estilai and Ray 1991). The triplods and the tetraploids are generally more vigorous, fast growing and produce more biomass and rubber than diploids (Kuruvadi *et al.* 1997). Therefore, triploids and tetraploids are used in cultivation (Thompson and Ray 1989).

2.3.3 Rubber biosynthesis

Rubber is synthesised and accumulated in over 2000 species of plants, representing seven families (Archer and Audley 1973). These families are Apocynaceae, Asclepiadaceae, Asteraceae, Euphorbiaceae, Loranthaceae, Moraceae, and Sapotaceae (Bealing 1969). Rubber biosynthesis in these plants is a side-branch of the ubiquitous isoprenoid pathway (Figure 2.4). Natural rubber is made almost entirely of isoprene units derived from the precursor, isopentenyl pyrophosphate (IPP). Also trans-allylic pyrophosphates are essential for rubber formation as they are used to initiate all new rubber molecules (Cornish *et al*. 1993). The elongation of the rubber molecule catalysed by the enzyme, rubber transferase (Backhaus 1985). The average molecular weights of rubber molecules vary considerably with plant species. Guayule and *Hevea* are unique among rubber producing plants in that they produce higher molecular weight rubber with an average molecular weight of ca $3\text{-}7 \times 10^5$ whereas other plants produce inferior quality rubber with a molecular weight of ca 5×10^4 or less (Backhaus 1985).

Effect of temperature

Guayule under cultivation exhibits a distinct seasonal cyclical pattern of alternate growth and rubber accumulation (Hammond and Polhamus 1965). Biomass production is greatest in the spring, summer and autumn months, and rubber accumulation is highest in the winter months. An outstanding feature of rubber formation in guayule is the stimulation of rubber biosynthesis by the exposure of plants to low night temperatures. Bonner (1943) demonstrated that the exposure of guayule plants to 27°C during the day and 7°C at night with an 8 hour photoperiod for 4 months in a controlled temperature glasshouse produced a 4-fold increase in rubber compared with plants grown at constant 27°C (day and night) with an 8 hour photoperiod. Bonner (1943) concluded that night temperatures of about 7 to 10°C were required for accumulation of rubber in guayule.

Ji *et al.* (1993) reported seasonal variations in rubber biosynthetic rate in guayule in the Chihuahuan desert. They found a very slow increase in rubber content during the warmer months (from 0.7% in August to 1.27% in October) and a very rapid increase during winter months (to 5.5% in December) when the night temperature was around 5 to 7°C.

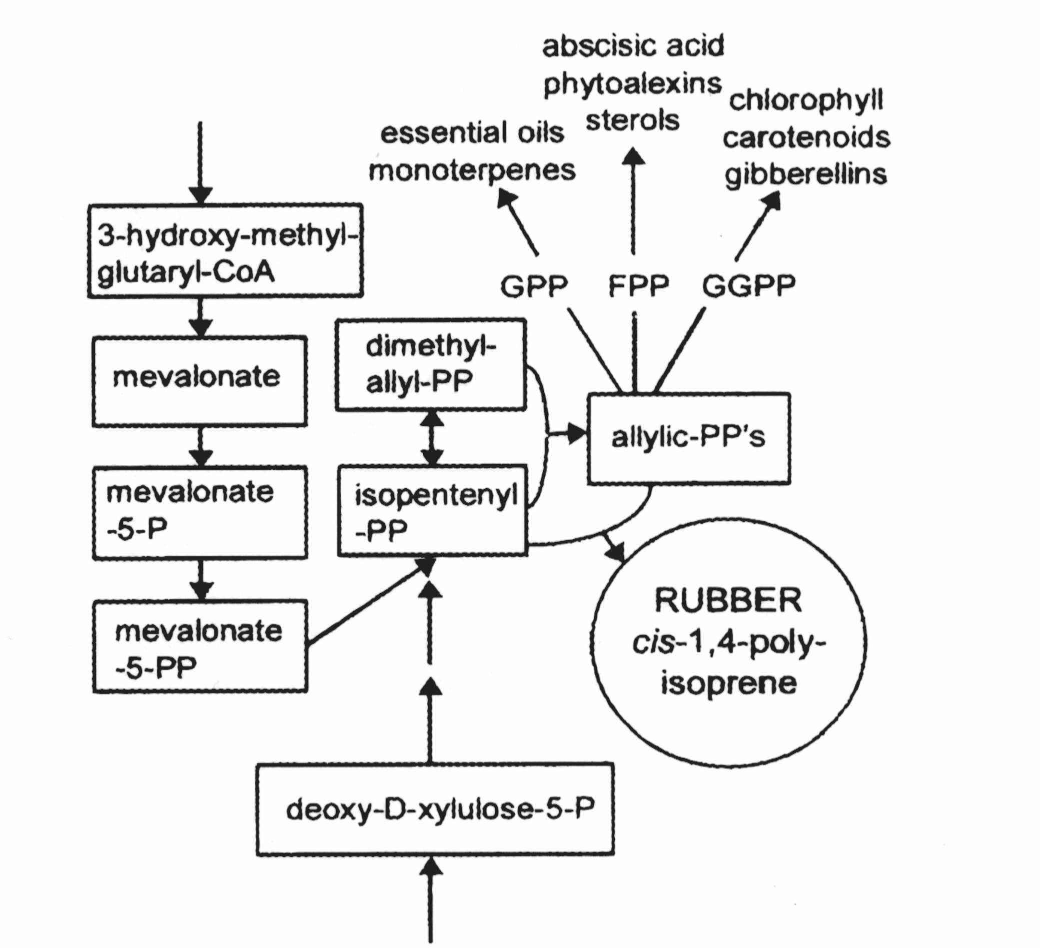


Figure 2.4. A section of the isoprenoid pathway illustrating the position of natural rubber biosynthesis (Cornish *et al.* 1993; Cornish 2001)

Sundar and Reddy (2001) discovered that three year old plants subjected to 60 cycles of a 30/15°C day/night temperature regime with 12-hour photoperiods in a control environment, had 80% more rubber transferase activity compared to the plants grown in the field with a 30/28°C day/night temperature regime. Cornish and Backhaus (2003) found that a cool night temperature of 20°C is enough to induce the rubber transferase activity in the synthesis of rubber.

These findings indicate that rubber transferase activity in guayule starts when the night temperature is around 20°C. Night temperatures below 20°C increase the rate of rubber biosynthesis until a maximum is reached at about 5°C. No data are available to explain the rate of rubber biosynthesis at temperatures below 5°C.

Effect of moisture stress

Hunter and Kelley (1946) first reported the effect of moisture stress on rubber. They conducted experiments in two soil types of sandy loam and silty clay. They maintained variable moisture stresses for a period of six months in the second year. They reported to have variable results for two sites. On the sandy loam soils, the highest rubber yields were given by plots with high level of moisture while on the silty clay loam, the highest rubber yields were produced by plots with lowest moisture levels. Conducting an experiment in fine loamy soils, Allen *et al.* (1987) found that soil moisture stress causes increased percentage of rubber but with reduced dry matter. They obtained increased rubber yield for plants with high moisture stress compared to plants had low or no moisture stress. Moisture stress for 70 days during the first year increased rubber content from 0.4% to 2.1% while plants with low or no stress had only 0.8% rubber content. Moisture stress also reduced plant dry matter, however, overall rubber yield reported to increase (3.6g versus 2.2g) with stressed plants. These results indicate the possibility of obtaining higher ruber yields by manipulation soil moisture (decrease irrigation) in fine textured soils (clay soils).

2.3.3 Ontogeny of rubber formation

Backhaus and Walsh (1983) reported that rubber formation in the stems of guayule first occurs in the cytoplasm of epithelial cells surrounding the resin ducts. They reported that rubber formation starts when plants are about 2.5 months old. However, this contradicts the finding by Cornish and Backhaus (2003) that the cold temperature stimulus that induces rubber biosynthesis either is not perceived or translated by guayule seedlings until they are at least 200-days-old. These inconsistent findings could be due to the effects of different environmental conditions.

Rubber particles did not appear in the vacuole at the initial stage (2.5 months) of development. The epithelial cells at this age are highly vacuolated and contain the usual complement of organelles (nuclei, plastids, mitochondria, microbodies, and rough endoplasmic reticulum). The rubber particles are relatively small at this stage, the largest one observed in sectional view being 1 μm in diameter. They are surrounded by a dense osmiophilic layer 400 A^o thick (Figure 2.5B) (Backhaus and Walsh 1983).

When the plants are 8 months old (during winter), parenchyma cells lying somewhat distal to epithelial cells show the accumulation of rubber particles almost exclusively in the cytoplasm, a few are observed in the vacuole. However, in parenchyma cells lying next to epithelial cells, the rubber particles appear in the vacuole as well as the cytoplasm. While the vacuole rubber particles are almost perfectly spherical, cytoplasmic rubber particles maintain an irregular, elliptical or globoid shape (Figure 2.5). These different shapes are maintained throughout the life of the plant. The rubber particles are roughly divided equally between the vacuole and cytoplasm in the parenchyma cells at this stage, but in the epithelial cells, the great majority of the rubber particles are located in the cytoplasm. During this period of development, the rubber particles enlarge, the largest being 3-4 μm in diameter. The number of particles also increases substantially compared with those found in younger cells (Backhaus and Walsh 1983).

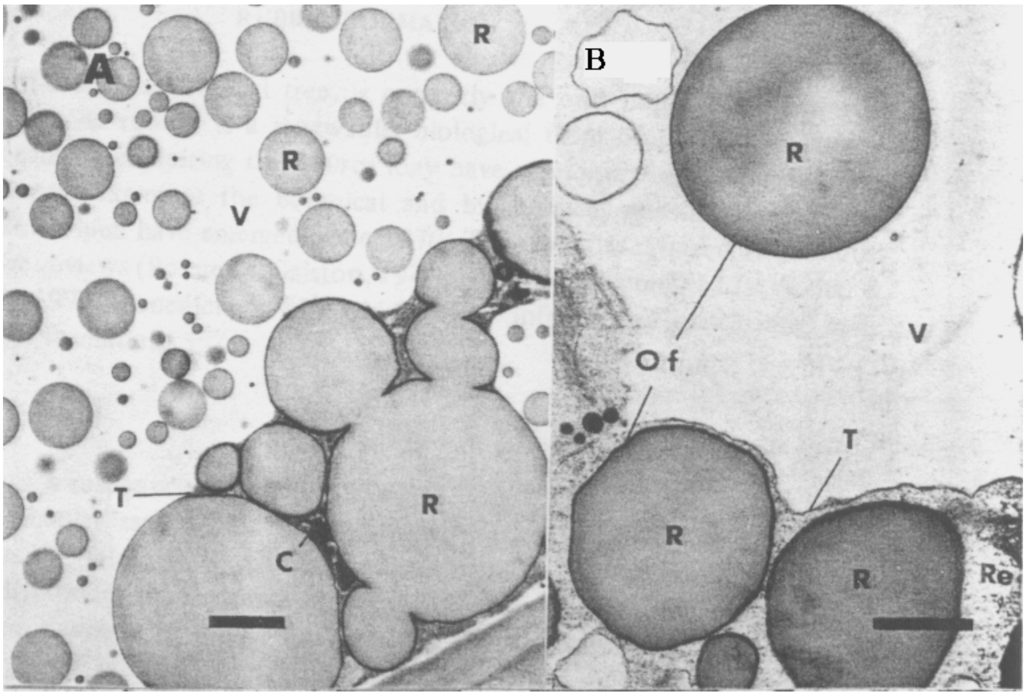


Figure 2.5 Electron micrographs of rubber bearing cells in guayule. (A). Final stage of rubber accumulation in cell from 2 ½ year old plant where a large proportion of rubber particles eventually accumulate in the vacuole. (B). Parenchyma cell showing difference in shape of rubber particles in vacuole and cytoplasm which contains rough endoplasmic reticulum. Rubber particles in both compartments are surrounded by a dense osmiophilic film. Horizontal bars = 1 µm. R= rubber, V = vacuole, T = tonoplast, C = cytoplasm, Of = osmiophilic film. Re = rough endoplasmic reticulum (Backhaus 1985).

In 3-year old plants, rubber accumulation proceeds further as cells mature, with more particles filling a greater volume of the available space in the cell. The largest individual rubber particles are no larger than they were in 8-months-old stems, but the total number of particles and the proportion of large particles per cell increase tremendously. When the guayule shrubs are harvested and extracted for rubber, both the epithelial cells and the parenchyma cells are highly vacuolated and contain little residual cytoplasm. Consequently, nearly all of the rubber particles occur in the vacuole and have the characteristic spherical shape peculiar to vacuole rubber (Backhaus and Walsh 1983).

2.3.4 Storage of rubber

Rubber is found in all plant organs, but the stem and the root contain most of the rubber of economic interest (Artschwager 1943). Curtis (1947) reported that leaf parenchyma contains about 0.3 to 0.5% rubber, however, Teetor *et al.* (2003) revealed that leaf has more rubber averaging about 1.7 to 2.0%. In general, in plants of harvest size (one year or older) the vascular rays of phloem and, to a lesser extent, those of the xylem contain by far the largest amount of rubber. Smaller quantities are found in the epithelial cells of resin canals, primary cortex, pith and xylem parenchyma (Artschwager 1943; Hammond and Polhamus 1965). In young plants, much of the rubber occurs in some of these latter tissues (Hammond and Polhamus 1965).

The bark is the principal site of rubber occurrence and it contains 75 to 80 percent of the total weight of rubber in the plant (Curtis 1947). Kuruvadi *et al*. (1997b) confirmed this result when they found wood and bark contained 17.6% and 82.4% of the total plant rubber respectively.

2.3.5 Function of rubber

Earlier it was believed that rubber serves as a food reserve in guayule. Later, Traub (1946) and Benedict (1949) demonstrated that this was not the case. Confirming this with *Hevea* and other plants, Archer and Audley (1973) and Backhaus (1985) reported that rubber in plants does not serve as an energy source. There is no evidence that plants have enzymes capable of degrading it and therefore, once rubber is formed it remains in the cell (Archer and Audley 1973). Despite speculation, the function of rubber in plants remains unknown (Hammond and Polhamus 1965; Backhaus 1985; Shin *et al*. 1999).

2.4 Adaptation

2.4.1 Climate

In its natural habitat, guayule occurs within a latitudinal range of 23^0 39’ N to 30^0 00’ N and an altitudinal range of 700 m to 2340 m (Nix 1986; Nakayama 1991; Anon 2000). Guayule is not a dominant species of the region and is scattered throughout 337,000 km^2 of the Chihuahuan Desert and surrounding regions. It is confined to calcareous slopes and well-drained soils (Naqvi and Hanson 1982). Precipitation in the region varies from 140 – 500 mm concentrated in the warmer months and the colder months are dry. The mean annual temperature in its natural environment is between 17 to 23^0C (Nix 1986) and the temperature extremes are $–23^0$C to 49^0C (Anon. 2000).

However, guayule has been cultivated and grown successfully under experimental conditions in rather different climatic environments in the USA, Australia, Israel, South Africa and India. Many of the experimental plantings have used irrigation to modify water regimes, although thermal and radiation regimes remained unchanged. Homoclimes of the natural distribution are most extensive in arid and semi-arid regions of inland Australia (Nix 1986).
The Emergency Rubber Project (ERP) and subsequent research in the USA have demonstrated that guayule can be grown successfully in climates that deviate (wet winters) from that of its natural distribution. Parallel research in Australia has concentrated efforts in somewhat similar wet winter/dry summer environments (Nix 1986).

2.4.2 Terrain and soil

In natural habitats, guayule occurs on a range of sites, but it favours stony, well-drained colluvial slopes and outwash fans. Valley floors are avoided. The best guayule stands occur on outwash fans on calcareous, medium to fine textured soils at the lower end of the altitude range (Anon. 1980).

In the natural habitat, guayule shows a distinct preference for soils developed on calcareous sediments with an alkaline to neutral pH. The soil profiles are commonly shallow (less than 30 cm) and stony. The density and cover of naturally occurring stands of guayule were negatively correlated with percent clay and soil phosphorus, over canopy cover and percent grass cover (Anon. 1980).

It has been reported that guayule requires moderate to well drained soils with no clay pan or other features that restrict penetration of water and air. Guayule cannot tolerate waterlogging. Presence of sand or sandy gravel horizons greatly reduces root development (Muller 1946), and results in very low shrub and rubber yields (Retzer and Mogen 1947). Salt content in the root zone should be less than 0.3%, otherwise yields are greatly reduced (Retzer and Mogen 1947). Texture throughout most of the profile should be medium to fine, so that an appreciable proportion of the stored soil water is held at high tension, which is considered to favour the formation of rubber (Hunter and Kelley 1946; Allen *et al*. 1987). Thus for high productivity guayule requires good agricultural soils. In suitable moist soils, roots penetrate to 2 m in 1 year and 4 m in 2 years (Muller 1946).

2.4.3 Biotic factors

Weeds

All available evidence indicates that guayule is not a strong competitor against weeds or other plant species, especially during the early establishment phase (Hammond and Polhamus 1965; Milthorpe 1984). Even in its natural habitat, competition from co-occurring species is postulated to be more important in limiting its distribution than abiotic factors (Anon. 1980). Thus, commercial cultivation of guayule is unlikely to be successful without cost-effective weed control programs (Milthorpe 1984).

Diseases

Around 20 pathogens are known to attack guayule, the most important are soil-borne organisms (Frangmeier *et al.* 1984). These diseases are closely associated with poor drainage and irrigation management (Siddiqui *et al*. 1982; Milthorpe 1984; Ferraris 1993). Important causal agents reported in the USA include fungal species of *Verticillium, Pythium, Phytophthora* and *Fusarium* spp (Siddiqui *et al*. 1982; Stone and Fries 1986; Ykema and Stutz 1991). *Phytophthora, Rhizoctonia* and *Fusarium* spp. were reported to cause root diseases in Australia (Milthorpe 1984; Ferraris 1993).

Pests

A large number of phytophagous invertebrates have been reported to feed on guayule, but few pose serious problems. Leaf-eating beetles, aphids and grasshoppers have damaged stands in the USA and rutherglen bugs, aphids, cutworms and *Heliothis* caterpillar infestations have been reported in Australia (Milthorpe 1984).

Vertebrate pests including rabbits and hares attack larger established plants during dry times, biting off leaves and chewing stems. Galahs and cockatoos rip out young seedlings, but it is unlikely that this would be a major problem for broad area commercial plantings (Milthorpe 1984; Ferraris 1986). Unlike other arid type plants, guayule is not affected by foraging animals. Generally, cattle and sheep do not harm the plant (Siddiqui *et al*. 1982).

2.5 Cultural requirements

2.5.1 Plant population

The Intercontinental Rubber Company used a plant population of 18,500 plants per ha with a 90 by 60 cm spacing to achieve maximum dry matter yield in four years after transplanting under dryland conditions (Hammond and Polhamus 1965). The plant spacing adopted during the Emergency Rubber Project (ERP) was 71 x 61 cm under dryland condition (23,000 plants/ha). Closer spacing of 71 x 51cm (27,600 plants per ha) increased dry matter production under irrigation after 2 to 3 years (Roberts 1946 cited in Hammond and Polhamus 1965). Increased rubber yield of 135 kg per ha was reported to obtain with 71 x 25 cm spacing (56,300 plants/ha) in 21 months when compared to 71 x 51 spacing (produced rubber yield of 113 kg/ha) under irrigation (Davis 1945 cited in Hammond and Polhamus 1965).

A population density of 27,000 plants/ha was used to estimate yields during the 1980s (Estilai and Ray 1991). Foster *et al*. (1993) reported that the plant spacing recommendation for transplants is 2 to 3 established seedlings/m^2, that is, about 27,500 plants/ha. Recent field trials in the USA used a row and plant spacing of 1 x 0.36 m (27,500 plants/ha) (Foster *et al*. 1999). However, no proper recommendation on optimum plant densities for guayule is available.

A plant population study was carried out during the late 1980s in Kingaroy, Queensland, using USDA varieties, N 565, 11591 and 11619. The plant densities were 0.9, 1.8, 3.6 and 5.6 plants/m^2 with 0.91 m row spacing. This study revealed that 3.6 plants/m^2 (36,000 plants/ha) produced the highest stem dry matter production for both irrigated and dryland conditions for a three year growth period (8 t/ha) (Ferraris 1993). A parallel study was conducted in Hillston, NSW using the same varieties at similar plant densities by Milthorpe (1994). He obtained poor stem and root dry matter yield of about 6 t/ha at a plant population of 52,600 per hectare. He also obtained increased stem dry matter by 0.11 t/ha for dryland and 0.13 t/ha for irrigated conditions for every 10,000 additional plants over the planting density range tested (9,200, 18,600, 36,700 and 52,600 plants per ha). However, the differences were not significant during the period of 3.6 years. Thus, further studies are required to determine the optimum plant densities and harvesting age and these need to test with new improved germplasm with high yielding and fast growing ability over a range of environmental conditions.

2.5.2 Nutrition

Guayule does not respond greatly to the application of nitrogen, phosphorous and potassium (Hammond and Polhamus 1965; Nakayama *et al*. 1991a). Also, nutrient deficiency symptoms are difficult to recognize and, thus have not been well established for guayule (Nakayama *et al.* 1991). However, nitrogen deficiency was found in soils with very low amount of clay (Davis and Abel 1944 cited in Hammond and Polhamus 1965). They further reported that guayule did not respond to application of phosphate fertilizer. Nutrient culture studies showed that growth and rubber accumulation decreased with decreasing available nitrogen and phosphorous (Bonner 1944; Bonner and Galston 1947). Bucks *et al*. (1985) increased dry matter yield with high nitrogen applications when water was not a limiting factor. They further observed that nitrogen application of more than 210 kg ha^{-1} was required to maximize the production over two years.

In a nutrient culture study, Bonner (1944) found that a high ratio of ammonium to nitrate nitrogen decreased both growth and rubber percentage. He further observed that guayule did not respond to sulphate deficiency. When calcium and potassium concentrations were low, growth and rubber accumulation were depressed, but this was negated if the magnesium concentration was high (Bonner 1944). Symptoms of boron deficiency were the most rapidly demonstrated of all the minor elements. Plants with such deficiency showed reduced growth and rubber concentration but could recover adequately when supplied with boron (Mitchell *et al.* 1944). Beckett *et al.* (1992) found that in addition to reduced plant growth, boron deficiency significantly reduced seed production and the percentage of germinable seeds. They revealed that the optimum concentration of boron in the soil for seed production was between 20 and 100 μM.

Salinity greatly increases the mortality of young guayule seedlings. Miyamoto *et al*. (1985, 1989) attributed this problem of salinity to high salt accumulation at the soil surface; this can drastically injure the hypocotyl of the emerging seedling. Transplants, on the other hand, are much more tolerant of salinity than emerging seedlings (Miyamoto *et al*. 1984). The plant can tolerate up to 0.3% salt in the soil, but salt content from 0.3 to 0.6% in the top 60 cm of soil greatly hinders growth or causes death (Retzer and Mogen 1946; Siddiqui and Locktov 1981).

2.5.3 Water use and irrigation

Guayule is tolerant to extreme water stresses of a long duration (Nakayama *et al*. 1991). Established plants undergo partial dormancy but can recover very rapidly when water becomes available (Ehrler and Nakayama 1984). Too little or excess water beyond field capacity affects germination of seeds and survival of young direct-seeded seedlings and young transplants (Hammond and Polhamus 1965). Excess water, especially during hot summer months, is harmful to guayule plants at all ages. Young plants are more susceptible to excess water that can cause problems with root disease, soil aeration, and weed competition.

Irrigation is one of the primary factors influencing the yield of rubber with soil moisture having a dominant effect (Retzer and Mogen 1947; Nakayama *et al.* 1991). Irrigation shortens the period until harvest and increases rubber yields. In arid environments, guayule yield increases were closely related to the amount of water applied (Nakayama *et al*. 1991). The annual water requirement for guayule was estimated to be in the range from 520 mm for the cool coastal zone to 910 mm for the hot arid zone (McGinnies and Mills 1980 cited in Hammong and Polhamus 1965). Bucks *et al*. (1985) found that water applications up to 2850 mm maximised the dry matter yield for a plant growth period of two years.

Water use efficiency for biomass production of irrigated guayule was in the range of 0.5 to 0.7 kg m^{-3} in Arizona, USA. The efficiency values were lower than for other crops grown in the area. However, guayule can survive under extreme water stress conditions that would kill other crops (Nakayama *et al*. 1991).

2.6 Plant breeding and germplasm

To become a commercial rubber crop, guayule must be economically competitive with *Hevea*. Research programs have prioritised genetic, cultural and processing constraints that need to be addressed to improve commercial potential of guayule. Of these factors, genetic improvement has been given the highest priority.

Annual rubber yields were very low and were between 220 to 560 kg ha^{-1} during the ERP. Plant breeding programs were successful in raising the annual rubber yields to between 600 and 900 kg ha^{-1} in the early 1990s. Estilai (1991) estimated that, depending on the rubber price and cost of production, yields of up to 1000 – 1500 kg ha^{-1} would be necessary for guayule to become a commercial crop in the U.S.

When guayule plant breeding research was re-established in the late 1970s, attempts were made to produce varieties for immediate use. Varieties 11591, 11604, 11605, 11619, 12229, N565 and N576 were planted in 1980 and, after the removal of the obvious off-types, the above varieties were jointly released by the USDA Agricultural Research Service and the Agricultural Experiment Stations of Arizona, California, New Mexico, and Texas (Estilai and Ray 1991). Both B 565 and 11591 were open pollinated selection from 4-265-I a 54 chromosome group from Durango, Mexico (Thompson and Ray 1989).

The latest development in plant breeding occurred in 1997 when six guayule germplasm, AZ-1 to AZ-6, were released jointly by the Agricultural Research Service, USDA and the Agricultural Experiment Station, The University of Arizona. These releases were selected for uniformity of plant appearance, fast growth, high resin content, and high rubber yielding ability. The original germplasm sources used in developing this germplasm were obtained from the National Seed Storage Laboratory, Fort Collins, Colorado (Ray et al. 1999). Some of the characteristics of new germplasm lines describe below;

AZ-1

AZ-1 was selected from USDA PI 478660. This germplasm was developed from a single plant selected after two years of growth for its increased rubber production. AZ-1 yielded more rubber (576 kg/ha) than N 565 (492 kg/ha) and 11591 (355 kg/ha) after 2 years of growth (Ray *et al.* 1999).

AZ-2 and AZ-3

AZ-2 and AZ-3 were developed from individual plant selections from USDA PI 478640. Plants of AZ-2 and AZ-3 produced more dry weight (0.78 and 0.47 kg/plant respectively) after 2 years of growth than N565 (0.27 kg/plant) or 11591 (0.28 kg/plant). Therefore, these two lines (AZ-2 and AZ-3) produced greater rubber yields (1235 and 611 kg/ha respectively) than N 565 (492 kg/ha) and 11591 (355 kg/ha) (Ray *et al.* 1999). The exceptional attribute of these lines is their vigorous early growth that could decrease time to initial harvest from three to two years (Ray *et al.* 1999).

AZ-4 and AZ-5

AZ-4 is a line mass selected from one of the original accessions and AZ-5 is a single-plant selection from the same source. These were selected for release based on their higher rubber content (7.5% and 7.2% respectively) and yield (789 and 675 kg/ha respectively) after 2 years growth compared with N565 and 11591. AZ-5 is morphologically more uniform than AZ-4 (Ray *et al.* 1999).

AZ-6

AZ-6 is a very uniform line developed through mass selection from one of the original selections. Plant dry weight (0.42 kg/plant), rubber content (7.1%) and rubber yields (808 kg/ha) were higher than N565 and 11591 after two years growth. The most significant attribute of AZ-6 was that it incorporated higher plant dry weight and rubber content into one line as well as faster growth potential (Ray *et al.* 1999).

2.7 Shrub processing and coproduct utilisation

Effective processing of rubber and non-rubber coproducts is essential for a viable guayule industry. Thus, considerable work has been done to improve processing of guayule and on the uses of coproducts.

2.7.1 Shrub processing

Rubber in guayule is suspended in the parenchyma cells, mainly in the bark, and must be released during processing. The most primitive method used to extract rubber was water flotation. This method was widely used from the beginning of the commercialisation of guayule until the end of the ERP (Hammond and Polhamus 1965). It was discarded due to difficulties in finding large quantities of quality water in semi-arid areas where guayule was grown, disposal of waste water with high sodium content, variable extraction efficiencies and quality control (Gartcide 1986). Recent research efforts were successful in developing a simultaneous extraction method to overcome the difficulties faced with the flotation method. A mixture of solvents, usually acetone and hexane or pentane was used in this method to extract rubber and resins. After the initial extraction, more acetone was added to coagulate the high molecular weight rubber. This method has been used successfully on an experimental scale to extract rubber by both Texas A & M University and Bridgestone/Firestone Corporation in Arizona (Wanger and Schloman 1991).

High quality latex

Allergy caused by *Hevea* latex has become a major health hazard since the 1980s with the increased use of latex products including medical gloves and condoms. Of the three types of allergy, Type I immediate hypersensitivity or IgE mediated anaphylactic reaction is life threatening and caused by proteins in the *Hevea* latex. It was reported that almost 10% of the population in the USA are allergic to *Hevea* latex (Anon 2000).

This life-threatening “latex allergy” to *Hevea* rubber products created an imperative need for the development of an alternative source of natural rubber (Cornish and Siler 1996). Thus, guayule has been tested and proven to produce a high quality natural rubber suitable for hypoallergenic products (Siler and Cornish 1994; Cornish and Siler 1996). Clinical trials on humans showed that even severely *Hevea*-hypersensitive patients had no reaction to guayule latex. Guayule rubber particles have much less protein than those of *Hevea*, and guayule latex can be produced with lower protein levels than even highly purified *Hevea* latex (Cornish and Siler 1996).

The Agricultural Research Service of the USDA has developed a procedure to produce hypoallergenic latex from guayule (Cornish 1996). This procedure is patented and licensed to the Yulex Corporation in the USA for the production of high value latex products (Wood 1997).

2.7.2 Uses of coproducts

It was estimated that 94% of the guayule biomass ends up as by-products of rubber processing. Therefore, the term ‘coproduct’ was used by Schloman and Wagner (1991). Research on these coproducts indicates that a potentially profitable industry may be developed from the resin, low molecular weight rubber (molecular weight of ca 5 x 10^4 or less), and bagasse. These investigations further indicated that their value may exceed the value of the rubber. Thus, the coproducts will play a critical role in the commercialisation of guayule (Wright *et al*. 1991).

Investigations have been conducted to exploit the economically possible coproducts of guayule. Resins or low molecular weight polyisoprenes, are one of the main coproducts and have been used successfully as wood preservatives (Nakayama *et al*. 2001), a feedstock for specialty chemicals (coatings and rubber additives), and as a high value fuel with no ash (Schloman and Wagner 1991).

The residue, bagasse, another useful coproduct, can be used to fuel the processing plants. Other potential uses of bagasse include use in pulp for papers, cardboard and other types of boards that are commercially manufactured (Siddiqui and Lucktov 1981; Nakayama *et al*. 2001). Today, bagasse is still being considered as a cogeneration fuel as well as a feedstock for gasification, conversion to liquid hydrocarbons, as a source of fermentable sugars, and as a fibre. These applications are not unique and are typical of other types of waste lignocellulose (Schloman and Wagner 1991).

2.8 Seed harvesting and processing

2.8.1 Seed harvesting

Guayule flowers and sets seed continuously when days are longer than 9.5 hours (Backhaus *et al.* 1989), temperatures are over 15ºC, and there is adequate soil moisture (Tipton *et al*. 1981). Because flowering lasts from late spring until autumn (Thompson and Ray 1989), maturity of seeds harvested at any one time varies. This requires several harvests to maximise seed yield and therefore needs gentle non-destructive harvesting to avoid damage to seed heads that are in the flowering or filling stage.

Guayule seed shatters readily at low humidity, making removal very easy but increasing the possibility of harvesting losses. Under high humidity, the seed becomes very difficult to dislodge and harvesting must be limited to late morning and early afternoon (Coates 1985). Complicating these harvesting problems are the relatively low yield of 1.1 kg/ha, the very small size of the seed (about 1,000 seed/g), and the low percentage of clean seed to bulk weight of material harvested (0.5 to 2.7 %) (Coates 1991).

Frequently, a high percentage of seeds are empty or non-viable with the normal range of viable embryos being 10 – 45% (Benedict and Robinson 1946; Thompson and Ray 1989). Harvesting and cleaning of seeds is an important aspect in commercialisation to reduce the percentage of empty seeds and to reduce the amount of chaff that contains germination inhibitors. Further, harvesting and processing costs of guayule seed need to be reduced to facilitate direct seeding technologies.

Several seed harvesters have been developed by the International Rubber Company, ERP and subsequent seed programs. Later, Coates (1985) developed an improved version of a vacuum-type seed harvester to overcome the difficulties faced with previous models. This model used suction and impact forces in harvesting seeds. A venturi air system provided the suction that reduced seed damage. Previous models in which seed passed through fans produced higher percentages of damaged seeds. Various attachments for harvesting heads and automatic head height control have greatly improved performance and ease of operation. A two-row harvester had a harvesting efficiency of 90 to 98 % for ripe seed under favourable harvesting conditions with a field capacity of 0.5 ha per hour (Coates 1985). However, conditions of extreme humidity reduced the harvesting efficiency of this machine (Coates 1990; 1991).

Later, two harvesters were developed to overcome the difficulties faced by the previous prototypes (Coates 1990). One employed combined multiple impacts and scraping forces to dislodge the more firmly attached seed. A collection surface passed under the plants and caught seed which was dislodged, but which failed to be gathered into the suction opening.

The second harvester was developed to collect shattered seed from the bottom of the furrows. This employed a suction device to collect shattered seed from the soil surface. Harvesting efficiency of these two harvesters was reported to be over 90%; however, suction of seeds from ground required additional cleaning processes and hence increased the cost (Coates 1990; 1991).

2.8.2 Threshing and cleaning

Harvested seeds usually contain, in addition to central clusters of male florets, dried leaves, flower stems, and other extraneous material. The floral attachments make the seeds too light for separation by air blast. Leaf particles and clusters of male florets may be so near the size of the seeds as to make screen separation ineffective (Hammond and Polhamus 1965). Thus seed cleaning is a challenging task.

The ERP developed an effective seed cleaning and threshing system for guayule. The system involves a sequence of operations: (1) preliminary cleaning, (2) threshing, (3) gravity separation, and (4) final cleaning (Hammond and Polhamus 1965).

Preliminary cleaning was accomplished by the combination of a power-driven shaker and a vibrating clipper cleaner. A shaker with 6 mm mesh wire was used to remove the coarse trash of stems and leaves. Then, a vibrating clipper cleaner with a three-screen riddle consisting of top screen with number 10 round holes for removing over size material, a middle screen of 12 by 2 mm slots for separating the clusters of disk florets from seed, and a bottom number 7 round hole screen for removing the fine trash was used.

Threshing was accomplished with a seed huller originally designed for hulling burclover. It consisted of a conical rubber-covered adjustable rotor turning inside a rubber-lined drum which removed the attached floral parts. The huller had the capacity to handle 200 kg (unthreshed weight) of seed material per hour.

The threshed material was then passed over a gravity separator to divide the material into two distinct streams, one containing chaff, empty achenes and dust; the other containing filled achenes and particles of stems and leaves. The machine had a capacity of 180 kg per hour and 95% of the filled seed was separated through proper adjustment of air, shaking speed and pitch.

Final cleaning for removing any heavy materials was accomplished by passing the filled, threshed seed through a vibrating clipper cleaner. This cleaner consisted of three screens of different perforations: a screen with 12 by 2 mm slots and middle and bottom screens with 2 mm and 1.25 mm round holes (Hammond and Polhamus 1965).

2.9 Seed dormancy

Dormancy is defined as a state in which seeds are prevented from germinating even under environmental conditions (moisture, oxygen, temperature and light) normally favourable for germination. If the minimum requirements for germination are lacking, the seed is described as quiescent (Bewley and Black 1994). This dormancy or quiescence must be relieved for seeds to germinate.

There are several physical and physiological mechanisms of seed dormancy, including both primary and secondary forms (Copeland and McDonald 2001).

2.9.1 Primary dormancy

Primary dormancy is the most common form of dormancy and it has two forms, exogenous and endogenous. Exogenous dormancy occurs when one or more essential germination components (e.g. water, oxygen and light) are not available to the seed. This type of dormancy is the most common and it generally related to physical properties of the seed coat (Bewley and Black 1994; Copeland and McDonald 2001). Environmental conditions during seed development and maturation influence the duration of endogenous dormancy. These environmental conditions include day length, moisture status, position of the seed in the fruit or inflorescence, age of the mother plant, and temperature.

Seed dormancy in some plants is regulated by a balance of endogenous growth inhibitors and promoters (Amen, 1968). The levels of these endogenous compounds are controlled by certain environmental stimuli such as light and temperature. A number of compounds have been isolated that can induce dormancy through their influence on metabolic inhibition (Copeland and McDonald, 2001). Phenolic compounds, coumarin and abscissic acid (ABA) are common germination inhibitors found to inhibit metabolic pathways in many plants. Of these, ABA is an extremely active inhibitor of seed germination and it is active at very low concentrations. ABA is known to inhibit the synthesis of enzymes that are important in the early stages of germination (Varty *et al.*, 1983).

Studying the effect of growth promoters and inhibitors in tomato seed, Ni and Bradford (1993) found that ABA and gibberellin dependent changes in seed dormancy and germination rates, whether due to endogenous or exogenous growth regulators, are based primarily upon corresponding shifts in the thresholds for radicle emergence. They further noticed that the thresholds determine both the rate and final extent of germination within the seed population.

2.9.2 Secondary dormancy

Non-dormant seeds may subsequently become dormant by exposure of the seed to conditions that unfavourable for germination. Temperature, light and darkness can all cause secondary dormancy (Evenari 1965). Secondary dormancy could be imposed in winter barley by exposure of the seed to temperatures between 50 and 90 ^{o}C (Frischknicht *et al*. 1961). Other causes such as excess amounts of water, chemicals, and gases sometimes cause secondary dormancy either by blocking a crucial point in the metabolic sequence leading to germination, or by changing the balance of growth-promoting versus growth-inhibiting substances (Copeland and McDonald 2001).

Guayule seed exhibits both exogenous and endogenous dormancy. Freshly harvested seeds believed to have a 6-12 months long dormancy due to impermeability of the inner seed coat to gas exchange. Fresh seeds reported to have two months endogenous or embryo dormancy (Benedict and Robinson 1946). Specific causes of endogenous dormancy have not been clearly identified. However, addition of gibberellic acid or exposure to light found to break embryo dormancy in guayule (Hammond 1959; Naqvi and Hanson 1980). Therefore, a main factor causing embryo dormancy could be due to the presence of phytochrome pigment that is converted into its active form (Pfr) when exposed to red light. The active form of phytochrome then induces gibberellic acid synthesis to activate germination as described by Yamaguchi and Kamiya (2002) for lettuce and *Arabidopsis* seeds. Substitution of light by external addition of gibberelic acid in the germination of guayule seeds supports the above claim (Hammond 1959: Chandra and Bucks 1986).

In addition to seed dormancy, guayule chaff contains germination inhibitors, which also have a negative influence on seedling emergence. Seven phenolic compounds have been extracted and identified from guayule chaff and the seed coat. These, in order of decreasing concentration, are p-hydroxybenzoic, protocatechuic, p-coumaric, ferulic, benzoic, vanillic, and cinnamic acids. All of them are germination and growth inhibitors. Apart from inhibiting germination, these compounds also retard seedling emergence and growth (Naqvi and Hanson 1982).

2.9.4 Overcoming dormancy

Seed coat imposed dormancy (exogenous and inhibitory chemical) can be overcome by mechanical or chemical removal of the seed coat or by softening of the seed coat (scarification) and leaching.

Mechanical scarification includes grinding seeds with abrasives, heating, chilling, drastic temperature shift, brief immersion in boiling water, piercing the seed coat with a needle or exposure to certain radio frequencies to alter seed coat integrity, permitting penetration of both water and gases. However, the duration of these treatments is critical, since prolonged treatment may result in seed damage while brief treatments may be ineffective (Copeland and McDonald 2001).

Chemicals also can be used to degrade the seed coat. Sulphuric acid, sodium hypochlorite and hydrogen peroxide have been used to scarify seeds (Emparan and Tysdal 1957; Naqvi and Hanson 1980; Hsiao and Quick 1984). Seeds must be thoroughly washed and dried after treatment otherwise reduction of germination may occur. Over scarification can injure the seed and therefore must be avoided. However, chemical scarification has not been commercially popular because the materials are hazardous to handle (Copeland and McDonald 2001). Other chemical scarification methods include use of selective seed coat enzymes such as cellulase and pectinase to degrade the seed coat (Lester 1985). Since many seed coats contain water-insoluble compounds that retard water entry into the seed, organic solvents such as alcohol and acetone have been used to dissolve and remove these insoluble constituents and permit imbibition (Rolston 1978).

Endogenous chemical dormancy can be alleviated through leaching out inhibitory chemicals. Leaching removes or dilutes the influence of inhibitor. Seeds that are dormant due to metabolic and osmotic inhibition can be overcome by leaching inhibitors using sufficient quantities of water (Copeland and McDonald 2001; McDonald 2000).

Exposure to light breaks dormancy in certain seeds including guayule. The presence of phytochrome pigment in seeds controls seed germination by controlling the balance of growth promoters and inhibitors. Exposure to light (red) converts phytochrome into its active form (Pfr) that induces the synthesis of growth promoters to stimulate germination (Copeland and McDonald 2001; Yamaguchi and Kamiya 2002).

Overcoming guayule seed dormancy

Benedict and Robinson (1946) carried out a series of experiments with hydrogen peroxide, perchloric acid, or nitric acid to break seed dormancy in guayule. They found that the oxidising action of the hypochlorite on the seed coat was responsible. Naqvi and Hanson (1980) were able to increase the germination of freshly harvested guayule seed from 52 to 75% by the addition of 1.0% NaOCl.

Emparan and Tysdal (1957) reported that both embryo and seed coat dormancy in guayule could be completely broken by light (3 to 4 days exposure) and temperature (optimum temperature 20 – 30°C) in addition to the hypochlorite (0.75%) treatment for freshly harvested seed. They further noticed that the effect of light and hypochlorite were additive. Hammond (1959) and Chandra and Bucks (1986) found that both embryo and inner seedcoat dormancy of freshly harvested seeds were completely broken by continuous exposure to daylight during a germination period of 3 weeks. Hammond (1959) further mentioned that gibberellin could be substituted for light to completely break both embryo and inner seed coat dormancy. Chandra and Bucks (1986) found that gibblerellic acid was very effective in substituting for light, however, their data showed some delay in achieving total germination (12 days compared to 8 days with light) than the light treatment. Addition of gibberellin also increased emergence of nondormant seeds (Hammond 1959).

Later, Naqvi and Hanson (1980) developed an improved method to break seed dormancy in guayule. This method involved a number of steps including soaking and washing in water to leach out inhibitory chemicals, oxidative treatment with sodium hypochlorite to make the seed coat permeable to gases (oxygen and carbon dioxide), and gibberellin, which acts as a partial light substitute. This procedure for freshly harvested seeds produced 100% germination under light and 70% in complete darkness (Naqvi and Hanson 1980).

2.10 Seed germination enhancements

The central objective of seed enhancement technology is to improve performance of seed germination under very specific regimes and planting equipment. Various techniques have been employed to assure this superior performance and most have found commercial application (Copeland and McDonald 2001).

2.10.1 Priming

Seed hydration or priming is a process of hydrating seeds to induce initial steps of germination using various protocols (and then redried) to permit routine handling (Copeland and McDonald 2001; McDonald 2000). When a seed is hydrated, physiological and biochemical changes occur and it results in an increased percentage and rate of germination, more uniform emergence, germination under a broader range of environments, and improved seedling vigour and growth (Khan 1992; Copeland and McDonald 2001). Several seed hydration procedures have been developed.

Soaking and misting seeds in water and redrying them before they complete germination is called hydropriming or prehydration, and is the simplest approach to hydrating seeds. Pregermination eliminates the variable effects of weather and soil conditions on germination to obtain a rapid and uniform emergence of seedlings from the soil. Seeds are first germinated under ideal conditions, usually in aerated water at temperatures of 18-20^{o}C, followed immediately by sowing (Currah et al. 1974; Darby and Salter 1976).

Osmopriming or osmoconditioning is the process of soaking seeds in aerated osmotica of low water potential to control the amount of water they imbibe. The process provides only sufficient moisture to initiate the early germination (Phase II of imbibition) but not sufficient to permit radicle protrusion (Phase III) (Akers and Holley 1986). Seed priming increases the percentage and rate of germination, expands the range of temperatures over which the seed will germinate, and increases the uniformity of stand establishment (Khan 1992).

Polyethylene glycol (PEG), KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, NaCI, glycerol, and mannitol have been used as osmotica. Mannitol and other low molecular weight osmotica are capable of being absorbed by the seeds, resulting in toxic effects in some cases (Bradford 1995). Today the most preferred osmoticum is PEG. It is a high molecular weight (6,000 to 8,000 daltons) inert compound. Large molecular size prevents it from entering the seed and creating toxic side effects associated with the use of other salts (Michel and Kaufmann 1973). A major disadvantage of PEG is that oxygen solubility is inversely related to its concentration (Mexal *et al*. 1975). Thus, when PEG is used in priming, the solution needs to be aerated (Akers 1990).

For optimum performance, seeds are soaked at 15ºC (Bradford 1986), in osmotica that possess a water potential of –0.8 to –1.6 Mpa (Khan 1992), for several hours (Guedes and Cantliffe 1980) to several weeks (Khan *et al*. 1980/1981). During osmopriming, beneficial compounds that break dormancy or otherwise improve seed performance can be incorporated into the osmoticum so that they can be taken up by the seed. Osmopriming has been primarily successful with small-seeded crops and less successful with large-seeded ones. Khan *et al.* (1978) found that osmopriming of lettuce, parsley, onion, soybean, pea and sweet corn with polyethylene glycol was effective in reducing the time required for germination and seedling emergence, and for establishing a uniform seedling stand.

Priming of guayule seed

Treatment method was developed to osmoprime guayule seed with PEG. This treatment was successful for improving germination, emergence and vigour of seedlings (Chandra and Bucks 1986; Bucks *et al.* 1986; Chandra 1991; Foster *et al.* 1999). Chandra (1991) developed the most recent method adding other improvements to further enhance the performance of direct seeded guayule. The recommended procedure included treatment of seeds with 25% polyethylene glycol, and 100 μm gibberellic acid, 0.05% potassium nitrate, and 0.1% tetramethylthiruram disulfide (thiram). The method was reported to increase germination to over 90% under light over a broad range of temperatures (15-33^{0}C). Osmopriming also invigorates seeds leading to the development of a greater percentage of normal seedlings that accumulate more dry matter in the shoot tissue (Chandra 1991). Chandra and Bucks (1986) found that osmopriming enhanced the viability and vigour characteristics of fresh guayule seeds. Foster *et al.* (1999) obtained a significantly higher germination of 76% (mean value from six lines) than raw seeds (56%) by using this method developed by Chandra (1991). Recent direct seeding trials reported to produce good establishment using this seed treatment method (Foster *et al*. 1999; 2001).

Solid matrix priming or matriconditioning is another approach to prime seed. Here solid carriers with low matric potentials are used for control hydration (Kubik *et al*. 1988; Taylor *et al*. 1988). The ideal carriers normally possess, a low matric potential, negligible water solubility, high water holding capacity, a high surface area, nontoxicity to the seed, and the ability to adhere to the seed surface (Khan 1992). Vermiculite and peat moss are two natural substances with these characteristics.

2.10.2 Biological seed treatments

Biological seed treatments make use of biological organisms to effectively control soil and seed pathogens. These are gaining increasing popularity because of safety concerns for humans and the environment as well as phytotoxicity problems associated with excess use of pesticides. In addition, biological seed treatments offer the potential for protecting the plant throughout its entire life cycle rather than just during the seed/seedling stage.

Application of furathiocarb and bendiocarb as polymer seed coatings to peas in the UK offered excellent control of thrips. Furathiocarb was also highly effective against *Sitona lineatus*. Metalaxyl and thiabendazole applied as a seed coating gave about 84% control of seed-borne *Ascochyta pisi*. This activity further improved the germination by the addition of thiram (Salter and Smith 1986). Ester *et al*. (1997) reported that leek seed coated with fipronil and imidacloprid gave a good control on thrips tabaci (Lind.). They further found that fipronil is the most effective insecticide and it did not cause phytotoxicity, whereas imidacloprid reduced and slowed germination.

2.10.3 Physical enhancements

Seeds vary greatly in size, shape and colour. In many cases, seed size is small or irregular, making handling and precision placement during planting difficult. In addition, seeds need to be protected from a range of pests that attack germinating seeds or seedlings. Seed coating technologies can be employed in both situations. Small quantities of nutrients can also be applied in close proximity to the seed to help minimise losses that occur when applied to the whole area or when applied as band (Scott 1989). Coating can facilitate mechanical sowing to achieve uniformity of plant spacing, and can act as a carrier for plant protectants, so materials can be applied in the target zone with minimal disruption to the soil ecology and environment (Scott 1989; Taylor et al. 1998).

Two main types of seed coatings are in commercial use: seed pelleting and seed coating. These range from surrounding the seed with a pellet to improve precision planting to coatings that contain products which protect the seeds against an array of pests or even modify the time that water is absorbed by the seed (Copeland and McDonald 2001). Pelleting is a process of deposition of a layer of inert materials that may obscure the original shape and size of the seed, resulting in a substantial weight increase and improved plantability (Taylor *et al.* 1998).

Many seeds are not uniformly round while others are so small and light. Both these cases hinder the accurate placement seed that affect germination and finally crop yield (Smith and Miller 1987). Pelleting of seed is recognised as an important addition to precision planting in many vegetables such as onion, lettuce, carrot, and various flowers (Copeland and McDonald 2001).

In pelleting, a mixture of fillers (clays, limestone, calcium carbonate, talc, vermiculite) and cementing additives (gum arabic, gelatin, methylcellulose, polyvinyl alcohol, polyoxy-lethylene glycol-based waxes) are combined to form the pellet. Compounds such as plant hormones, micronutrients, microbes and fungicides could also be added to enhance seed performance (Taylor and Harman 1990; Copeland and McDonald 2001).

Seed coating is one of the most economical approaches to improving seed performance. A seed coating is a substance that is applied to the seed but does not obscure its shape. Often, the purpose of the coating is to apply substances such as fungicides, insecticides, safeners, micronutrients, and other compounds directly to the seed. This enables the seed to avoid specific stresses anticipated in certain planting environments.

The ideal seed coating polymer should, be a water-based polymer, have a low viscosity range, have a high concentration of solids, have an adjustable hydrophilic-hydrophobic balance, and form a hard film upon drying (Rushing 1988 cited in Copeland and McDonald 2001). These traits should lead to excellent plantability and provide for excellent germination under variable environmental conditions.

One of the major benefits of physical seed enhancements (pelleting and coating) is that they are placed directly on the seed and in the immediate vicinity of the germinating seedling. Therefore, less chemical is required compared to broadcast or furrow applications with far less cost, while avoiding environmental damage from excess pesticide use (Scott 1989).

Scott and Blair (1988) obtained increased plant growth and dry matter yield by using phosphorus seed coating for establishment of phalaris (*Phalaris aquatica* L.) and lucerne (*Medicago sativa* L.). However, phosphorus coating was reported to delay seedling emergence slightly. Greipsson (1999) reported significantly higher seedling establishment of *Poa pratensis* on barren land at Geitarsandur, Iceland, when seeds were coated with the growth regulator, cytokinin, and diatomaceous earth, compared to uncoated seeds and seed coated with water retaining polymer.

2.11 Field establishment

Of the many cultural practices, plant establishment was recognised as the most critical in guayule production. This is because of the high cost involved in the current widely used and reliable method of transplanting glasshouse-grown seedlings (Bucks *et al.* 1986; Foster and Moore 1992; Foster *et al.* 1999). The cost of establishing a crop by transplants in the USA was estimated be about US$ 1,600 (Foster *et al*. 2001) and in Australia it was estimated to be about A$ 1,800 per ha (based on 27,500 plants/ha) (personal com. K. Jackwitz, 2003). The method involves growing seedlings in a glasshouse for one month. The seedlings are then hardened to adapt to harsher field conditions for one to two months. Two to three months old seedlings are then ready to transplant in the field.

High levels of seed dormancy, small size seed, low seedling vigour and poor establishment are challenges in developing direct seeding methodologies for guayule. However, development of direct seeding methodologies has been recognised as one of the most effective ways to reduce the cost of production in guayule (Bucks *et al*. 1986; Foster and Moore 1992; Foster *et al.* 1999). Direct seeding not only reduces the production cost but also negates the difficulties with the current transplanting method. Development of direct seeding method for guayule requires a thorough understanding of all factors relating to germination, emergence and growth of seedlings and, precision placement of seed that described below;

2.11.1 Germination

To the seed physiologist, germination is the elongation and emergence of the embryonic axis, usually radicle (or rarely the epicotyl) through the seed. Germination begins with water uptake by the dry or quiescent seed (imbibition) thus causing a rise in metabolic activity and ends with the start of elongation of the embryonic axis (Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994).

Moisture

Moisture is the most important prerequisite for triggering germination. It is essential for enzyme activation, breakdown, translocation, and use of reserve storage material. Very low levels of moisture allow dry or quiescent seeds to maintain a minimum level of metabolic activity to assure their long-term survival in the soil and during storage (Copeland and McDonald 2001).
The moisture requirement for germination varies among species, however, field capacity moisture is about optimum for many species. Too much moisture (beyond field capacity) can cause the soil or germinating medium to become saturated and deprive the seed of oxygen, leading to death. Water uptake by seeds during germination has been described using a three-stage model consisting of imbibition, active metabolism and hydrolysis and visible germination (Chong *et al*. 2002).

Oxygen

Most seeds require an adequate supply of oxygen during germination. Oxygen is required for respiration to oxidise starches, fats, and other food reserves, and its utilisation is proportional to the level of metabolic activity (Hopkins 1999). If the oxygen concentration is reduced substantially below that of air, germination of most seed is retarded.

In general a germinating medium or seedbed should be loose, friable, and well aerated. Deep planting is unfavourable to germination because the oxygen supply may be restricted or seedlings may be unable to reach the surface, especially if the soil or medium is hard or compacted (Chong *et al*. 2002).

The influence of carbon dioxide on seed germination is usually opposite to that of oxygen. Most seed fail to germinate if the CO_2 partial pressure is increased over 0.03% of air; however, a decrease usually does not hinder germination (Copeland and McDonald 2001).

When moisture is adequate, the next most important requirement for germination is a suitable temperature range. Temperature affects the rate at which water is imbibed as well as the rate of metabolic process such as the translocation of nutrients and hormones, cell division and elongation, and other physiological and biological processes (Chong *et al*. 2002).

Temperature

The response to temperature depends on a number of factors, including the species, variety, growing region, quality of the seed, and duration of time from harvest. In general, temperate region seeds require lower temperatures than tropical-region seeds. The optimum temperature for germination of most seeds is between 15 and 30^0C. The maximum temperature for most species is between 30 and 40^0C (Copeland and McDonald 2001).

As for many seeds, temperature is a crucial factor for germination of guayule seeds. Emparan and Tysdal (1957) reported that temperatures which approximate a 20°C - 30°C range are best and, if they rise much above 30°C or fall below 15°C, germination is lowered. Ojeda and Trione (1990) observed that the optimum temperature for guayule seed germination is between 20 and 25°C. They confirmed the finding of Emparan and Tysdal (1957) that temperatures above 30°C lower germination. Untreated seeds germinate poorly in the dark over a narrow temperature range of 15 - 19°C (Chandra and Bucks 1986; Chandra 1991). Continuous exposure to light will stimulate germination over a broader temperature range of 20 – 30°C. Recently it was discovered that 20°C is the optimum temperature for laboratory germination of guayule seed under light (Marcos 2002, unpublished data).

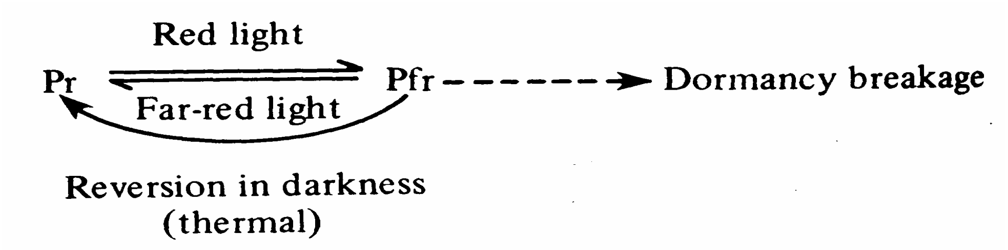
Foster et al. (1999) reported that direct seeding was most successful when the soil temperature exceeded 15°C. The upper critical temperature for germination of seed under light was found to be 33°C. However, temperatures above 25°C greatly reduced seed germination under dark conditions (Chandra 1991). From these findings, it can be concluded that temperatures ranging from 15 to 25°C are optimum for germination of direct seeded guayule. However, seed osmoprimed with polyethylene glycol (PEG) was found to exhibit a broader temperature optimum of 15 to 30^oC for germination (Chandra and Bucks 1986).

Light

In addition to moisture, oxygen, and favourable temperature, certain species also require light to activate germination. A study carried out the light response of several hundred species revealed that almost half of the species investigated responded to light (Copeland and McDonald 2001). Light requirement or photosensitivity of seeds is of particular significance to many small-seeded species (Chong *et al*. 2002). Photosensitivity has been discovered in seeds of lettuce, tobacco, birch, peppergrass, pine, elm and shepherd’s purse (Copeland and McDonald 2001) and *Arabidopsis* (Yamaguchi and Kamiya 2002). Emparan and Tysdal (1957) and Hammond (1959) reported photosensitivity in guayule.

The mechanism of light control in seed germination is similar to that controlling floral induction, stem elongation, pigment formation in certain fruits and leaves, and radicle development of certain seedlings. Both light intensity and quality (colour or wavelength) influence germination (Copeland and McDonald 2001).

Although, the influence of light intensity on different species varies greatly, a light intensity range of 1080 – 2160 lux is adequate for germination of most seeds (Copeland and McDonald 1995). Reaction to light (photosensitivity) is mediated through phytochrome, a protein pigment that absorbs either red or far-red light (Bewley and Black 1994; Copeland and McDonald 2001; Chong *et al*. 2002). In its most concentrated form, it appears blue, is faded by red light, and reintensified by far-red light (Copeland and McDonald 2001). Phytochrome is converted to Pr (red light-absorbing form) by 730 nm (far-red) light (Chong *et al*. 2002). The far-red absorbing form, Pfr (induced by exposure to red light - peak at 660 nm) is the biologically active form that functions by inducing the synthesis of enzymes essential for germination (Copeland and McDonald 2001; Yamaguchi and Kamiya 2002). Exposure to relatively high temperature also causes the reversion of Pfr to Pr in some seeds. This was discovered with lettuce seeds (Bewley and Black 1994).



Photosensitivity of guayule seed has long been known (Emparan and Tysdal 1957; Mammond 1959). However the requirement of light quality or intensity has not been clearly defined for guayule. Exposure to light during imbibition and the early stages of germination increases the percent germination of guayule seed (Eamparan and Tysdal 1957; Hammond 1959; Chandra 1991). Emparan and Tysdal (1957) and Hammond (1959) reported that exposure to light for 3 to 4 days during germination, plus oxidation brought about by using 0.75% NaOCl, completely breaks the dormancy of freshly harvested guayule seed.

Hammond (1959) further reported that gibberellin substitutes for light in completely breaking both embryo and inner seed coat dormancy. He also reported that gibberellin increased emergence of nondormant seeds under soil cover. Naqvi and Hanson (1980) and Chandra and Bucks (1986) found that gibberellic acid effectively replaced the light requirement for germination but cannot be completely substituted. In an experiment carried out using osmoprimed seed (using PEG) and addition of gibberellic acid during priming, Chandra (1991) found 94% germination under light compared to 80% without light after seven days.

Yamaguchi and Kamiya (2002) found regulation of gibberellin biosynthesis by phytochrome in germinating lettuce and *Arabidopsis* seeds. They reported that the effect of light in lettuce and *Arabidopsis*, at least in part, activates the GA 3-oxidase, which catalyses the final biosynthetic step to produce bioactive gibberellins (Yamaguchi and Kamiya 2002). This explains the reason for efficient substitution of light in germination of guayule seed by addition of gibberellic acid. Interactions between light and temperature are known for seed of some species. It is clear that the response to each can sometimes be increased, decreased, or changed qualitatively by the other, while in other cases it cannot. For example, photosensitivity may be overcome by alternating low and high temperatures. In general, light and temperature have interactive effects on germination, however, the exact mechanism of the light and temperature interaction remains unknown (Copeland and McDonald 2001).

Externally applied chemicals can also interact with light and temperature. Many nitrogenous compounds, including cyanide, nitric acid, ammonium salts, urea, thiourea, and particularly potassium nitrate (10-20 mM solutions), have been found to stimulate the germination of photosensitive seeds (Hendricks and Taylorson 1972).

2.11.2 Emergence and vigour of seedlings

Guayule produces very small and fragile seeds. The average length and width of a seed is about 2.5 mm and 1.8 mm, respectively (Tipton *et al.* 1981), and one kilogram contains about one million seeds (Naqvi and Hanson 1980; Siddiqui *et al.* 1982; Coates 1985; Bucks *et al.* 1986). Seed size is one of the primary factors limiting the development of direct seeding technologies. It affects seedling emergence (Naqvi and Hanson 1980; Thompson and Ray 1989), vigour and growth (Bucks *et al.* 1986; Chandra 1991), and precision planting.

Naqvi and Hanson (1982) reported emergence and survival of guayule seed treated with NaOCl and gibberellic acid under optimum glasshouse condition of 14/10 hrs of light and dark and 26°C to 21°C day and night temperatures. They found that seed having an initial laboratory germination of 65%, produced seedling emergence and survival of 28% and 25% after 7 and 17 days respectively. Bucks *et al.* (1986) obtained similar results in a field experiment in a sandy soil in Arizona. Using osmoprimed seed with PEG, they were able to obtain maximum seedling survival of 15% three months after direct seeding by using seeds that had 33% laboratory germination. They conducted their trial under irrigation with water having electrical conductivity of 1.4 dS/m and reported that seedling vigour and salt tolerance are the two main problems to be solved before direct seeding can be recommended.

Experimenting with several untreated seed lots, Chandra (1991) found that development of normal seedlings varies from 11% to 87%. He further reported that osmopriming of these seed lots with PEG increased the percent normal seedlings from between 1% to 81% with one exception that produced an increase of 178%.

Foster and Moore (1992) obtained highly variable results in two field trials conducted in Texas on a very gravelly loam soil. They planted their trials in four consecutive months from July to October 1988 and 1989 and used osmoprimed seeds from two cultivars. In one experiment they used cultivar 11591 with a laboratory germination of 63%. They obtained maximum survival of 16% after two months (1st Nov.) for seeds planted at a depth of 10 mm. The other trial was planted in 1989 using the cultivar 12229 with seeds having 90% germination. The maximum establishment obtained in this trial after two months was 72%. Interestingly both produced the best establishment for August plantings (late summer).

Foster *et al*. (1999) in Texas, USA reported better results for establishment. They used osmoprimed seeds from six guayule lines planted at 10 mm depth at a rate of 100 seeds/m. Laboratory germination of treated seeds ranged from 56% to 92%. Establishment of these lines after two months from direct seeding ranged from 40% to 68% (Foster *et al*. 1999). However, adequate data on this trial was not available to compare with other results.

Chandra and Bucks (1986) and Chandra (1991) reported that direct seeding of guayule, even with seed of high germination, remains unreliable due to poor seedling emergence and subsequent mortality. The above data support this claim by producing highly variable results for germination, emergence and establishment. There is a requirement for further research to develop a reliable method of direct seeding of guayule to lower production cost.

2.11.3 External factors influence on direct seeding of guayule

Seedbed and planting depth

Good establishment of tiny and fragile guayule seeds essentially requires a well-prepared seedbed and precision planting. Whitworth (1981) reported that good quality seed, fine and uniform seedbed and precision planting were essential for successful direct seeding of guayule. Therefore, land preparation should pay special attention to obtain the required soil tilth. Shallow planting of seed is another requirement for better establishment due to the influence of seed size on emergence and also due to requirement of light in germination. Naqvi and Hanson (1980) reported that seedling emergence in a glasshouse decreased from 81% to 19% for large seeds (0.866 g/1000 seeds) and from 53% to 12% for medium seeds (0.783 g/1000 seeds) when seeding depth was increased from zero to 18 mm. It was reported that seed could not be covered by more than 6 mm of soil without a decrease in establishment (Emparan and Tysdal 1957; Hammond and Polhamus 1965; Naqvi and Hanson 1980). However, seed osmopriming with polyethylene glycol (PEG) under light and addition of gibberellic acid during the treatment increased seed germination and establishment under field conditions. Foster and Moore (1992) obtained better establishment when osmoprimed seed was planted at 10 mm depth compared to surface planting. Osomopriming was also reported to increase the rate and uniformity of germination and, addition of gibberellic acid during treatment increased seedling emergence and growth. Further, the treatment improved the performance of seed and seedlings in a wider range of temperatures (15 to 30°C) and field conditions (Chandra and Bucks 1986; Foster *et al.* 1999). Recent direct seeding trials with osmoprimed seeds achieved good results for germination and emergence when they were planted at 10 mm depth (Foster *et al.* 1999; 2001).

Irrigation and salinity

Irrigation is important for maintaining soil moisture during germination and early growth of seedlings to avoid desiccation. However, the frequency and amount of irrigation should also be monitored carefully to avoid the damping-off diseases associated with soil-borne fungi (Mihail *et al.* 1991). Miyamoto *et al.* (1985) suggested that water suction needed to be kept below 0.1 Mpa for optimum emergence in sandy soils. Among the other requirements for direct seeding of guayule are prevention of crusting of the soil surface and protection of the seed and seedling from excessive salinity (Miyamoto *et al.* 1984, 1985; Bucks *et al.* 1986). Miyamoto *et al*. (1984) reported 47 to 57% of seedling emergence in the field when furrow irrigated with water of 0.8 dS/m (510 ppm), while emergence declined to 17% when irrigated with water of 4.5 dS/m (2,880 ppm). They further reported that survival of emerged seedlings was 10% and zero with waters of 0.8 and 4.5 dS/m respectively.

Soil temperature

Soil temperature is a crucial factor for better performance in direct seeded guayule. Temperatures below 15°C and above 30°C affect germination of guayule seed (Emparan and Tysdal 1957; Chandra 1991). More importantly temperatures above 30 °C decrease seedling vigour and increase seedling mortality. Bucks *et al*. (1986) reported that seedling mortality under high temperature and semiarid field conditions was a major problem in direct seeding of guayule.

Weed control

Guayule is a slow growing plant and hence is not a good competitor against weeds (Milthorpe 1984; Foster *et al.* 1993). Seedlings produced only 10 mm top growth and 50 mm root growth two weeks after emergence (Miyamoto and Bucks 1985). Bucks *et al*. (1986) reported that the growth of direct seeded guayule seedlings during the first three months was extremely slow with root growth of about 100 mm, top growth of less than 75 mm, and total fresh weight of under 100 g (Bucks *et al*. 1986). Direct seeding requires frequent irrigation for germination and growth of seedlings and this also increases germination and growth of weed seeds. Therefore, field establishment of guayule through direct seeding requires effective weed control through mechanical or pre-plant or pre-emergence herbicides. Foster *et al.* (1993) found that direct seeded guayule shows adequate tolerance to dimethyl 2, 3, 5, 6-tetrachloro-1, 4,-benzenedicarboxylate (a pre-emergence herbicide used to control annual grass and broadleaf weed).

Pest and diseases

Flea beetles (*Epitrix sp.)* have reported to attack young seedlings in USA. Damping off diseases were associated with soil borne fungi *Pythium sp., Phytophthora sp., Fusarium sp.,* and *Rhizoctonia sp.*, prevalent under moist conditions as the seedling become established (Mihail *et at*. 1991). Pest such as aphids and grass hopers were reported to attack young transplants and nursery seedlings. These could be possible pests for direct seeded guayule.

2.12 Production potential in Australia

Large areas of semi-arid land are available in Australia, but a combination of factors such as climate, terrain, soil and biotic conditions determine the areas potentially suitable for guayule rubber production. Infrastructure, social and economic factors will further narrow the selection of specific areas most favourable for development (Nix 1986).

Guayule requires well-drained soils with medium to fine textured subsoils, slightly acid to alkaline reaction, and with low salt content. The main soil types that appear to have these requirements are the red earths, and well-drained duplex and clay soils (Nix 1986). Based on climate and soil factors, it was estimated that the gross area of potentially suitable soils is approximately 6 million hectares (Stewart and Lucas 1986).

2.12.1 Climate

Nix (1986) reported that a very large area in Australia satisfies the temperature requirements of guayule. The homoclime of the natural distribution cuts a broad swath across central Australia, but mean annual rainfall falls below the threshold for dryland production. Therefore, a major proportion of this available land would require irrigation for commercial guayule production (Nix 1986).

A summer rainfall zone, 100 – 200 km in width and 2000 km in length, stretches from north of Clermont in central Queensland to the Hunter Valley and Central Tablelands and slopes of New South Wales (Nix 1986). Citing the research experience of Paterson and Jones in South Africa, Nix (1986) reported that the summer rainfall zones having annual mean rainfalls of 600 – 700 mm have shown results superior to those of the winter rainfall zones. He also indicated that the sites that closely match in terms of climatic attributes are the Northwest Slopes of N.S.W. and the Western Downs and Maranoa Regions of southern Queensland (Nix 1986).

A zone of wet winters and dry summers extends southwards to cover most of the southern arid inland, the southern wheat/sheep zone and the major irrigation areas of the Murray-Darling system. Much of this broad zone has annual rainfall totals that fall below the 325 mm threshold for rainfed cultivation but a substantial part of the wheat/sheep belt meets this requirement (Nix 1986).

A broad zone in central eastern Australia is of particular interest because of its transitional status between summer and winter rainfall regimes. Patterns of episodic recharge and draw down of soil water in both summer and winter increase the number of wetting and drying cycles. This may be of significance for processes of rubber accumulation, particularly when such cycles occur in the warmer season when temperatures are favourable for growth of guayule.

In those parts of the indicated zone where winters are wet and summers are dry, temperature and water regimes are out of phase for guayule. Under rainfed conditions, favourable combinations of water availability and temperature occur only for relatively short periods in autumn and again in spring (Nix 1986).

Since guayule is a desert plant, it can tolerate extremely high water stresses of a long duration (Nakayama 1991). In its natural habitat, guayule is reported to grow in areas with a very low annual rainfall of 250 mm (Anon. 2000). Similarly, Australia has vast area of lands of very low annual rainfall. Therefore, there is potential to grow guayule in these low rainfall areas where other crops cannot survive.

2.12.2 Terrain and soil

Agro-climatic analysis indicates that the most favourable environments for dryland guayule rubber production should be in sub-coastal and inland regions of eastern Australia. Most of the areas with suitable climate, terrain and soils in New South Wales and southern and central Queensland are already under crop or sown pasture. Therefore, guayule needs to be economically competitive with existing crops in the region (Rawlins 1986).

According to the requirement of guayule, deep, structured and massive red earths that have slightly acid to neutral or alkaline profiles should be particularly suitable. Duplex soils that have sandy or loamy surface horizons over clay subsoils, without mottling in the upper part of the subsoil and/or without prominent bleaching above this, should be suitable for guayule. Some of these duplex soils must be excluded because of strongly acid surface and/or subsoil horizons and others because of moderate to high salt content. These duplex soils occur extensively throughout the indicated region and include the well-known red brown earths of the cereal belt. Cracking clay soils present problems in terms of slow rates of internal drainage and potential waterlogging. This may not be so critical in sloping to gentle undulating terrain (Nix 1986).

It is difficult to predict soil suitability in Australia for guayule until much more extensive field testing is completed. However, soils that have strongly acid profiles and evidence of water logging should be avoided (Milthorpe 1984; Nix 1986).

2.12.3 Biotic factors

Weeds

Weed control is likely to be the major biotic constraint in all the recommended areas in Australia. In winter rainfall zones, guayule stays dormant and weed species adapted to lower temperatures make vigorous growth. In this region, *Echium plantagineum* (Paterson's Curse) and *Arctotheca calendula* (Capeweed) are reported to be the more common weed species (Milthorpe 1984). In the summer rainfall zone, guayule would grow adequately during the summer, but fast growing weed species especially grasses will pose problems. Thus, efficient and economic weed control measures have to be taken if guayule to be grown commercially.

Diseases

The second major problem is likely to be root diseases caused by soil pathogens (Ferraris 1993). Areas that have been previously cultivated for annual crops are likely to cause more problems. The areas already infested with soil-borne diseases should be treated or avoided. Poor drainage and irrigation management is the basic causes of these soil-borne diseases. Thus, these diseases can be controlled by improving drainage through terracing, mounding, hilling up or bed and furrow techniques. Crop rotation and fallowing also help in controlling these diseases (Ferraris 1986).

Pests

Although a number of vertebrate and invertebrate pests have been reported, they have not presented serious problems in experimental planting and where such problems occurred, standard control measures can be taken (Milthorpe 1984).

2.13 Conclusions

The major gaps that emerge from this literature review are listed below. These need to be addressed in order to develop guayule production system for commercial rubber in Australia.

Improved guayule lines with high yielding ability have been developed in the USA. Performance of these lines, which is determined by genetic and environment interaction have not been evaluated in Australia. Therefore, these improved guayule lines needs to be evaluated in Australian environmental conditions.

Lack of knowledge in some areas of seed dormancy especially on embryo dormancy is a constraint to the development of appropriate low cost establishment method.
An economically sound and practically feasible establishment method is yet to be developed for commercial production of guayule. High levels of seed dormancy and, low levels of seedling emergence and establishment are a major constraint in developing direct seeding methodologies for guayule. Thus, research is required to improve establishment of guayule by direct seeding. Very little work has been reported on the use of physical seed enhancement techniques for guayule (coating) that could improve the performance of direct seeded guayule. Thus, investigation into use of these technologies could improve the commercial potential of guayule.

3. Germplasm evaluation

3.1 Introduction

Guayule is yet to become a commercial rubber crop. Germplasm lines developed until late 1980s did not become commercial varieties mainly due to their low rubber yields. Research conducted with these lines in the USA concluded that annual rubber yields need to be increased up to 1000 to 1500 kg/ha depending on rubber prices (Estilai, 1991). Investigations have also been carried out in Australia during 1980s. Performance of some of the lines developed during World War II was evaluated during the period. These researchers concluded that guayule was not economical with then available cultivars due to lower annual rubber yield (Milthorpe 1984; Ferraris, 1993).

Therefore, with the beginning of new era of guayule research in mid 1970s, plant breeding research programs have been given priority to develop high yielding lines. These efforts have been successful in releasing new lines with high yielding ability in the U.S. The Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) and The University of Arizona jointly released six improved lines of guayule that have high yielding ability with fast regrowth after harvest in the U.S.A. (Ray *et al.*, 1999).

Newly released lines produced significantly higher rubber and resin yields compared to existing lines in environmental conditions in the USA (Ray *et al.*, 1999; Coates *et al.*, 2001). The objective of this study was to evaluate the potential of these improved guayule lines in different environmental conditions in Australia.

3.2 Materials and method

3.2.1 Field sites description

Two field sites were selected within the implied region for guayule in Queensland, Australia. One site was located at Gatton and the other was located at Chinchilla. These sites were chosen for their different environmental conditions (rainfall, temperature and soil type). Description of these two sites is summarized below;

Gatton:
The Gatton site was located at the Gatton Campus of the University of Queensland (latitude 27°33’S, longitude 152°20’E, altitude 89 m) in the Lockyer Valley, approximately 80 km west of Brisbane. The soil type was a Lawes Black Earth, self-mulching cracking clay with less than 0.5% slope. The pH of the soil was 7.9 and soil organic carbon content was 1.2%. Soil was low in nitrogen (17.7 mg/kg of Nitrate Nitrogen) and had adequate levels of phosphorous and potassium (189 mg/kg of P and 1.09 meq/100 g of K). The site receives an average annual rainfall of 763 mm that is summer dominant with 68% of rain usually falling between October and March (Powell, 1982). However, the study period was comparatively dry and received only 1372 mm of rain for 29 months of plant growth from September 2001 to January 2004 (Figure 3.1).

Summers are warm to hot with maximum temperatures of 28-33°C, although temperatures up to 40°C can occur. During winter, minimum temperatures vary from 6°C to 10°C. Frosts are possible during June, July and August. The mean values of minimum and maximum temperature during the study period are shown in Figure 3.2.

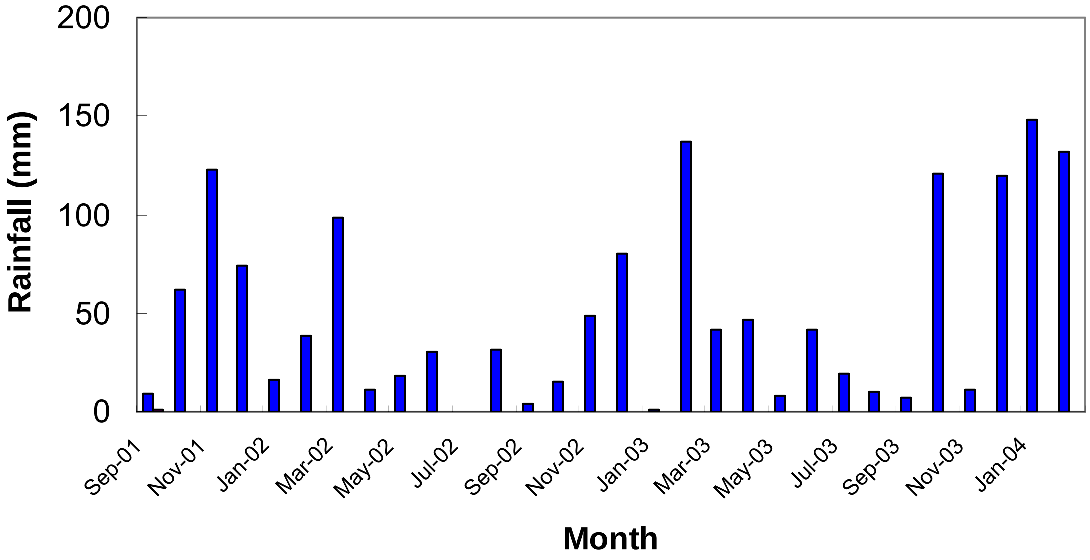


Figure 3.1 Monthly rainfall at Gatton during the study period

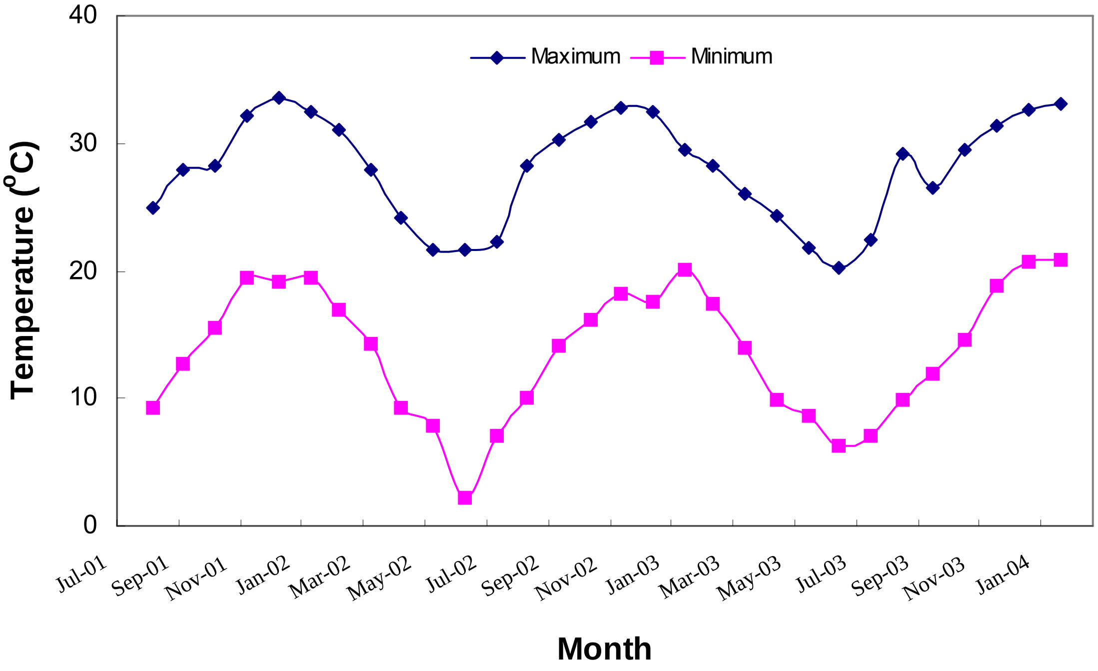


Figure 3.2 Monthly mean values of maximum and minimum temperatures of Gatton site during the study period

Chinchilla:
The Chinchilla site was located in the agricultural plots at the Chinchilla State High School (latitude 26°57’27’S, longitude 150°51’48’E, altitude 320 m), approximately 300 km northwest of Brisbane. The soil type was a flat, very deep, reddish brown sand (Maher, 1996). The pH of the surface soil was 6.5. Subsoils become more acidic with depth. The soils are non-saline and non-sodic. Low water holding capacity was a major limiting factor in crop production. Soils were low in organic carbon (0.4%) and almost all nutrients except potassium.

The site receives an average annual rainfall of 633 mm that is summer dominant with 75% of rain usually falling between October and March. However, the study period was comparatively dry and received only 1041 mm of rain for the 31 months of the trial, from March 2002 to September 2004 (Figure 3.3).

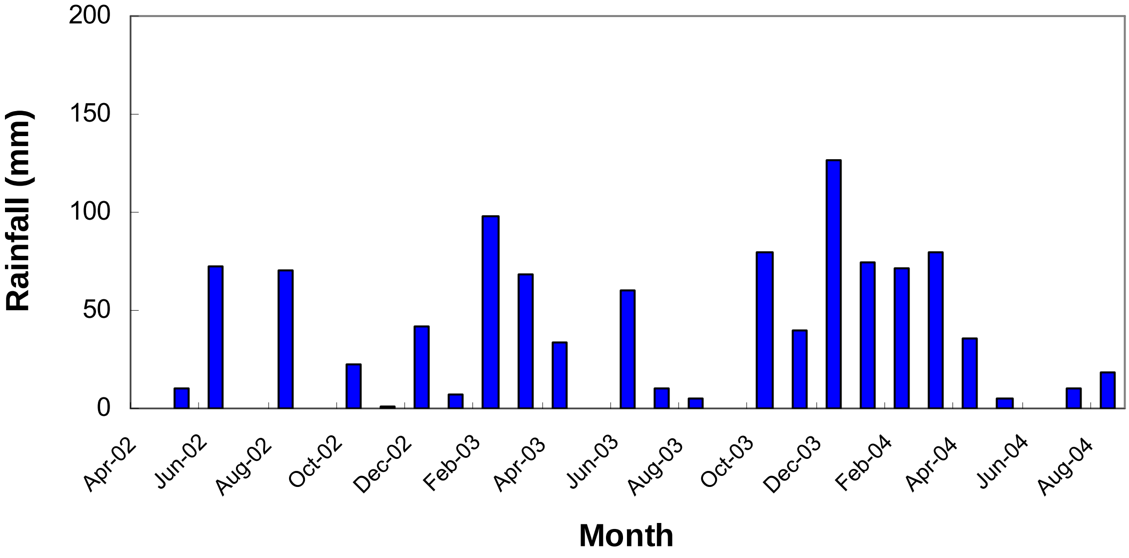


Figure 3.3 Monthly rainfall at Chinchilla during the study period

Temperature data at Chinchilla was not available. Therefore, the closest matching data were obtained from Dalby (about 80 km away towards Brisbane). These data show that summers of this particular area are warm to hot with maximum temperatures of 30-33°C. During winter, minimum temperatures are usually from 3°C to 7°C. Frosts are possible during June, July and August. The mean values of minimum and maximum temperature in Dalby region during the study period are shown in Figure 3.4.

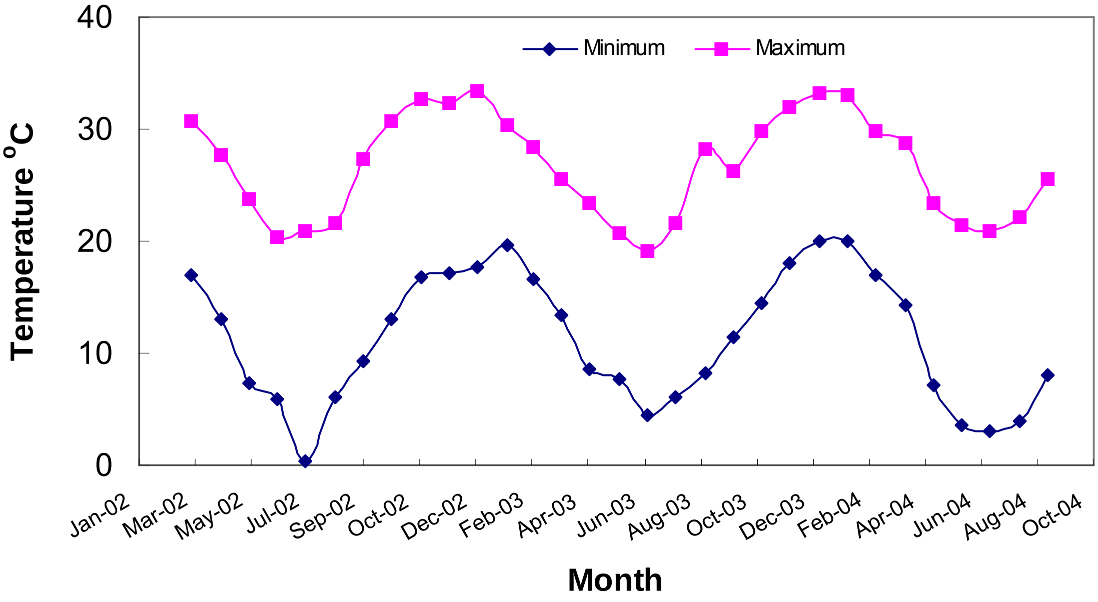


Figure 3.4 Monthly mean values of maximum and minimum temperatures of Dalby region during the study period

3.2.2 Germplasm lines

Guayule germplasm released jointly by the ARS-USDA and The University of Arizona were evaluated (Ray *et al*., 1999). This germplasm consisted of six improved guayule lines, AZ-1, AZ-2, AZ-3, AZ-4, AZ-5 and AZ-6. Seed from six guayule germplam lines were obtained from the Agricultural Research Service of the United States Department of Agriculture (ARS-USDA). Guayule lines N 565 and 11591, which were developed during World War II under the Emergency Rubber Project, were used as controls. All lines were grown at both sites except the line AZ-4 at Gatton due to unavailability of seed.

3.2.3 Raising seedlings and transplanting

Seed was treated to break dormancy using the method of Naqvi and Hanson (1980). This method includes washing and soaking of seeds in distilled water for 8 hours followed by a 2-hour treatment with a solution of equal parts of gibberellic acid (200 ppm) and 0.25% NaOCl. Seeds were then rinsed with demineralised water to remove residual NaOCl. Treated seeds were sown into polystyrene trays containing 1:1:1 media of peat, vermiculite and perlite. Slow release fertilizer, mini osmocote plus, was also added to the media at the rate of 2g per litre. These trays were then placed in the greenhouse and mist irrigation was provided every day during the first week and then the frequency of irrigation was gradually reduced. Seedlings were grown in these trays in the nursery for one month. Thereafter, some changes were adopted for the two trial sites as follows;

Gatton:
Month old seedlings were transferred to 12.5 cm diameter polyethylene bags containing 2:2:1 media of saw dust, pine bark and sand. One cubic meter of this media also contained 3 kg of osmocote plus, 2 kg of nutricote, 1.33 kg of coated iron, 1.2 kg of Saturaid, 1.2 kg of dolomite and 1.33 kg of slow release nitrogen fertilizer. When the seedlings were two months of age they were transferred to a shade house for hardening. In September 2001, three months old plants were transplanted in the field.

Chinchilla:
Establishment of the Chinchilla plots occurred six months later than the Gatton trial because additional seed from the ARS-USDA was needed. Sowing started in February 2002. One month later, seedling trays were transferred to a shade house for hardening. In March 2002, two months old seedlings were transplanted in the field.

3.2.4 Experimental design

The experimental design used in both trials was a randomised complete block. Seedlings were transplanted onto plastic mulched raised beds with trickle irrigation. The Gatton trail was replicated three times with a plot size of four rows at 1.5m spacing. Each row consisted of 10 plants at 0.35m spacing. A guard row was planted between plots. The Chinchilla trail was replicated four times with a plot size of five rows at 1.5 m spacing. The number of plants in a row and plant spacing was the same as for Gatton.

3.2.5 Irrigation and fertilizer application

Gatton:
Plots were irrigated using a trickle system to supplement soil moisture. A total of 279 mm of irrigation water was applied during the 29 months of crop duration. Including rainfall (1372 mm), the trial received a total of 1651 mm of water at a rate of 683 mm/year. Fertilizer was applied on two occasions; the first was during field preparation when 23 kg N/ha (ammonium sulphate) was incorporated into the soil. A second application of 40 kg N/ha (urea) was made seven months after transplanting through the irrigation water.

Chinchilla:
Plots were irrigated using a trickle system. The amount and frequency of irrigation was much higher than Gatton due to the low water holding capacity of the soil. A total of 1143 mm of irrigation water was applied to the plots over 31 months. Therefore, the trial received a total of 2184 mm of water (including a rainfall of 1041 mm) at a rate of 845 mm/year. Slow release fertilizer (8-9 months active), osmocote plus, that contain 16% N, 3.5% P, 10% K, 2.4% S, 1.2% Mg, 0.4% Fe, 0.05% Cu, 0.02% B, was applied just before bed forming at a rate of 250 kg/ha.

3.3 Measurements and data collection

3.3.1 Plant establishment and growth

The total number of established seedlings in each plot was recorded at the end of the fourth week. Plant growth was monitored by recording height and width at regular intervals. Five plants from each plot were randomly selected to monitor growth. Plant height was measured by taking the vertical distance from the ground level to the top of the plant. Plant width was recorded by measuring the widest part of the plant (measurements were taken horizontal to the ground surface and perpendicular to the row). Some lines had high variability in plant growth compared to others. Therefore, the variability was measured by recording the plant height and width. The methods adopted in evaluating plant uniformity were:

Gatton:
Variation in plant height and width for each line was determined from the coefficients of variation (CV) at 19 weeks after transplanting. These values were calculated by measuring the plant height and width of all 18 plants in the two middle rows (excluding border plants). Death of plants due to an outbreak of a root disease prevented the determination of plant uniformity at the end of the study period.

Chinchilla:
Two distinctly different patterns of plant growth were observed in Chinchilla. Three of the improved lines (AZ-1, AZ-2 and AZ-3) and two controls produced comparatively uniform plant canopies. Three other lines, AZ-4, AZ-5 and AZ-6 had non-uniform plant growth. These lines comprised two distinctly different plant types with large and small canopies. Therefore, two data sets, one from large plants and the other from small plants together with the number of plants in each category were recorded to determine plant uniformity.

3.3.2 Harvesting and dry matter analysis

Methods of harvesting and determination of dry matter were similar for both trials: however, some changes were adopted in the timing and number of harvests. Two harvests, one at 17 months, and the other at 32 months, were carried out at Gatton. The initial harvest was carried out in early spring (September 2002). Four plants were clipped at the ground level to analyse dry matter, rubber and resin production. The second harvest occurred in late summer (February 2004). The second harvest was initially planned for spring 2004. However, the harvest date was advanced due to the outbreak of root disease (*Rhizoctonia solanii*) and plant death following heavy summer rainfall. Plants were harvested immediately after plant death of some plots was observed. At this harvest, six randomly selected plants were clipped at ground level.

The Chinchilla trial was harvested only once when plants were 33 months of age (spring - September 2004). The trial had five lines with comparatively uniform plant canopy (AZ-1, AZ-2, AZ-3, N 565 and 11591) and three with two distinctly different types (small and large) of plants (AZ-4, AZ-5 and AZ-6). Of the uniform lines, six randomly selected plants were clipped at ground level. Four randomly selected small plants and two large plants were selected from lines with variable plant growth. Root sampling on two blocks was also carried out in similar way and they were uprooted to a depth of 25 cm using a tractor mounted implement. Four randomly selected plants from a plot were uprooted from lines AZ-1, AZ-2, AZ-3, N 565 and 11591. For AZ-4, AZ-5 and AZ-6, two large plants and two small plants were uprooted. Dry matter and, rubber and resin content were determined on root samples.

All harvested plants were weighed for dry matter while subsamples were selected for rubber and resin analysis. Initially, leaves plus immature and dead branches from the harvested plants were removed from the plant and the remaining stems and branches were then stored in a cold room until they were chipped using a garden shredder to facilitate drying and sampling. The entire biomass from harvested plants from each plot was oven dried at 60°C to estimate the total plant, stem and branch dry matter yields. Roots also were washed, chipped and oven dried at 60°C to estimate the dry matter yield.

3.3.3 Rubber and resin extraction and analysis

Representative samples of clipped plant materials (about 50 to 75 g) were ground using a Retsch grinding mill. These ground samples were passed through a 2.36 mm round holes sieve and the samples were stored in a freezer until they were used to extract rubber and resin. These samples were analysed for rubber and resin contents using the method of Black *et al.* (1983) and revised by USDA, ARS (per. com. V.H. Teetor). Rubber and resin yields were calculated using the product of stem and branch dry weight and rubber and resin contents. Sampling procedure adopted for the two trials was;

Gatton:
At 17 months, four samples, one from each of the four plants harvested from a plot, were taken to extract rubber and resin. At the final harvest, four samples from three plants (one had duplicate samples) were used for the analyses.

Chinchilla:
Plant variability in some lines lead to adoption of two different methods of sampling. Lines AZ-1, AZ-2, AZ-3, N 565 and 11591 had a comparatively uniform plant canopy, therefore, a composite sample from two plants in a plot was selected for rubber and resins analyses. The ground samples were then subdivided into four for rubber and resin analysis (four samples/two plants).

Two composite samples, one from two small plants and the other from two large plants were chosen from lines AZ-4, AZ-5 and AZ-6. Each sample then was subdivided into two for rubber and resin analysis. A composite sample was drawn from the roots of four plants from lines AZ-1, AZ-2, AZ-3, N 565 and 11591. The samples were then subdivided in to three for rubber and resin analyses (three samples/plot). The roots of two smaller and two larger plants were used in lines AZ-4, AZ5 and AZ-6. A composite sample was drawn from each category of plants and later it was subdivided into three for analyses.

3.3.4 Data analysis

Variances on establishment, plant dry matter yield, rubber and resin contents and yields at two sites were analysed separately using Minitab (version 14) general linear model (GLM) procedure. Whenever analysis showed up significant difference, least significant difference (LSD) was computed at 0.05 probability level to compare the means of each parameter.

Means of each and every parameter for two sites was ranked to check whether they were following similar trend or have interactions. Dry matter yields, rubber content and yields showed similar trend while interactions were noticed for resin content and yields. Therefore, across site analysis were carried out only for dry mater yields, rubber contents and yields using Minitab (version 14).

Mean values for plant height and width was computed together with coefficient of variance to determine plant variability at Gatton using Minitab (version 14). Dry matter, rubber and resin contents and yields data were recorded separately for large and small plants of AZ-4, AZ-5 and AZ-6 at Chinchilla. Those results from large and small plants for each and every line were compared using 't' test procedure.

3.4 Results – Gatton

3.4.1 Seedling establishment

The survival rate of transplants was over 95% for all lines except for line AZ-5 which had a significantly lower survival rate of 83% than all other lines (Table 3.1).

3.4.2 Plant growth and variability

At the early stage of plant growth, the new lines grew faster (height and width) than the controls (Figure 3.5 and 3.6, Plate 3.1). Of the five new lines, AZ-2 and AZ-3 maintained the greatest height and width during the first year of growth. The relative differences of plant height and width among lines became narrower after the first year. However, some of the improved lines (AZ-1, AZ-2 and AZ-3) continued to produce larger plant canopies throughout the period of study. Final plant height and width of AZ-5 and AZ-6 also were higher than line N 565 but not for line 11591.

Table 3.1 Seedling establishment four weeks after transplanting

Line	**Mean Plant Establishment (%)**
AZ-1	98.3 a*
AZ-2	100.0 a
AZ-3	99.2 a
AZ-5	83.3 b
AZ-6	97.5 a
N 565	100.0 a
11591	95.8 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey’s simultaneous test.

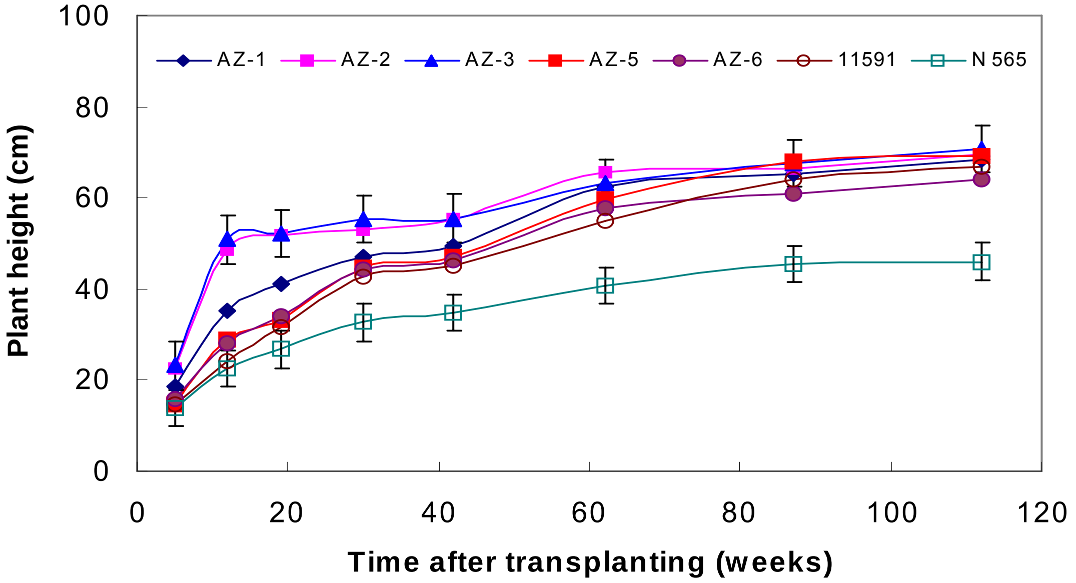


Figure 3.5 Plant height of guayule lines from transplanting to age 32 months at Gatton

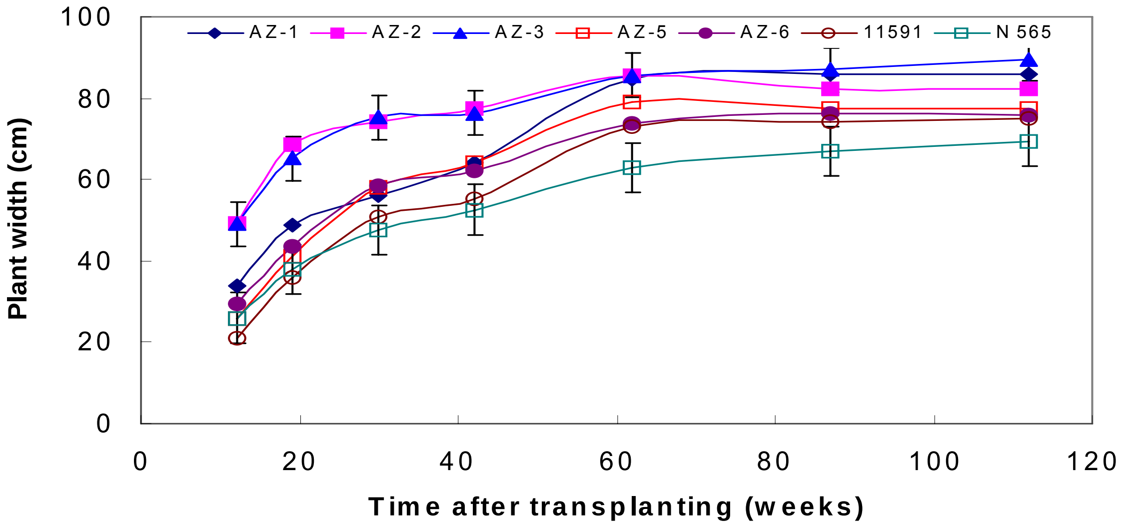


Figure 3.6 Plant width of guayule lines from transplanting to age 32 months at Gatton

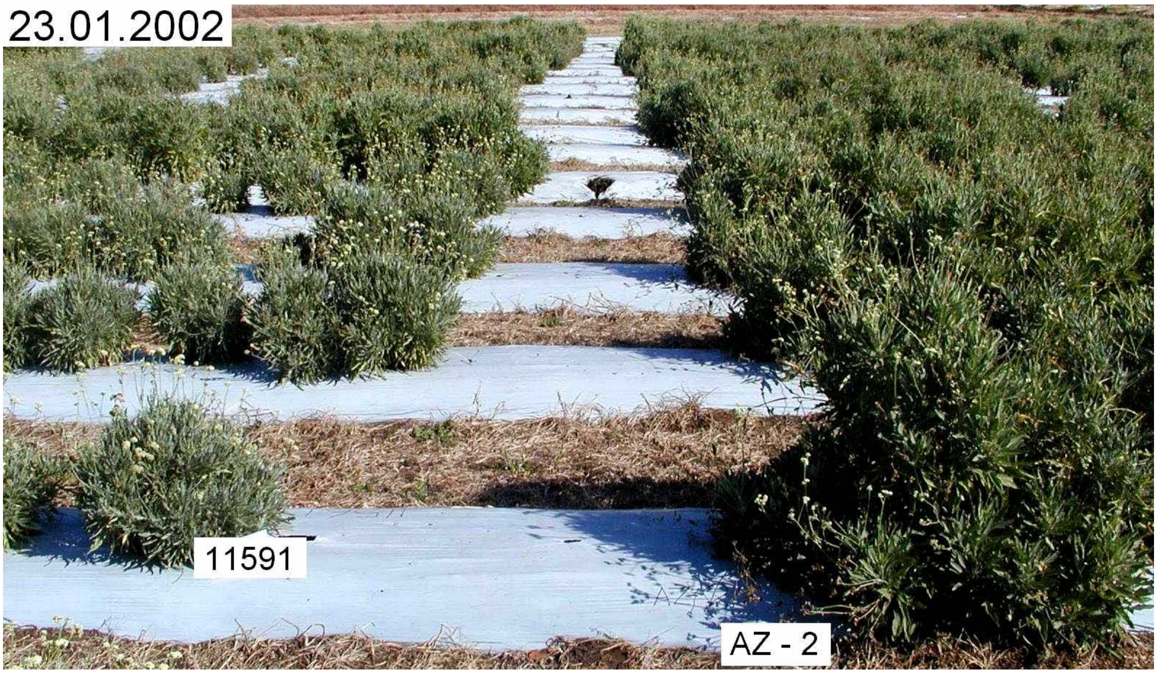


Plate 3.1 Plant canopy development of one of the promising new line (AZ-2) compared with an old line (11591)

Plant height and width of the new lines generally were greater than those of 11591 and N 565 at 19 weeks (Table 3.2). Coefficients of variation for plant height and width for lines at 19 weeks after transplanting are summarized in Table 3.2. Among the new lines, AZ-3 was least variable with a CV for plant height of 16% and for plant width of 18%. AZ-2 was next best with a CV of 18% for height and a CV of 24% for width. AZ-1 was highly variable for height (CV = 25%) and width (CV = 41%). This trend of plant variability was observed throughout the 32 months of the trial.

3.4.3 Biomass production

Dry matter production at 17 and 32 months showed significant differences among lines (Tables 3.3 and 3.4). In general, the new lines produced or tended to produce more dry matter than controls.

Table 3.2 Mean plant height and width, and coefficients of variation at 19 weeks after transplanting.

Line	Sample size	Plant height		Plant width	
		Mean (cm)	CV (%)	Mean (cm)	CV%
AZ-1	48	41.3 b	25.4	48.8 bc	40.6
AZ-2	48	52.3 a	18.3	68.5 a	23.7
AZ-3	47	50.9 a	16.4	65.2 a	17.8
AZ-5	41	33.0 c	27.2	41.2 cd	44.1
AZ-6	47	34.2 c	18.7	43.7 c	28.4
11591	47	26.7 d	16.2	37.8 d	21.2
N 565	48	31.6 cd	10.5	35.7 d	17.6

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey’s simultaneous test.

At 17 months, total plant dry matter (excluding roots) of AZ-1 (13.1 t/ha) was significantly greater than N 565 (7.1 t/ha) and 11591 (8.2 t/ha). AZ-2 (11.4 t/ha) and AZ-3 (10.9 t/ha) produced the second and third highest dry matter yields; however, they were not significantly greater than the controls. Stem and branch dry matter yields showed similar trends to total dry matter yields. The stem and branch dry matter yield of AZ-1 (8.3 t/ha) was significantly higher than N 565 (4.6 t/ha) and 11591 (5.1 t/ha). AZ-2 (7.7 t/ha) had a significantly greater stem and branch dry matter yield than N 565, but not 11591. The increases in stem dry matter of AZ-1 and AZ-2 were in the range of 51 to 80% compared with 11591 and N 565.

New lines also produced higher plant (excluding roots) and, stem and branch dry matter for the final harvest (Table 3.4). These lines produced a minimum increase of plant dry matter of 3.7 t/ha and stem and branch dry matter of 2.8 t/ha compared to controls. Stem dry matter yield of AZ-1 (14.3 t/ha) and AZ-6 (14.6) was significantly greater than N 565 (8.9 t/ha) and 11591 (9.4 t/ha). Stem dry matter production of AZ-2 (13.2 t/ha), AZ-3 (12.2) and AZ-5 (13.9 t/ha) tended be higher than the controls but the differences were not significant. Increases in stem dry matter of AZ-1 and AZ-6 compared to N 565 and 11591 were in the range of 52 to 63%. Stem and branch dry matter yield increased from harvest 1 to 2 was highest for lines AZ-5 and AZ-6 (Figure 3.10). They both produced more than 100% yield increase during the period.

Table 3.3 Mean of agronomic and chemical characters of lines at 17 months of age at Gatton

Line	**Plant dry matter (t/ha)**	**Stem & branch dry matter (t/ha)**	**Rubber content (%)**	**Rubber yield (kg/ha)**	**Resin content (%)**	**Resin yield (kg/ha)**
AZ-1	13.1 a*	8.3 a	7.4 ab	611 a	8.6 ab	725 a
AZ-2	11.4 ab	7.7 ab	7.4 ab	567 ab	8.7 ab	663 ab
AZ-3	10.9 ab	7.0 abc	6.9 b	478 ab	8.7 ab	604 ab
AZ-5	9.8 ab	6.8 abc	8.4 a	565 ab	8.0 bc	541 abc
AZ-6	8.9 ab	6.1 abc	8.2 ab	503 ab	7.7 bc	463 bc
N 565	7.1 b	4.6 c	8.2 ab	379 b	9.4 a	434 bc
11591	8.2 b	5.1 bc	7.4 ab	383 b	6.5 c	331 c
LSD$_{(0.05)}$	**4.4**	**2.7**	**1.4**	**207**	**1.6**	**238**

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

Table 3.4 Mean of agronomic and chemical characters of lines at 32 months of age at Gatton

Line	**Plant dry matter (t/ha)**	**Stem & branch dry matter (t/ha)**	**Rubber content (%)**	**Rubber yield (kg/ha)**	**Resin content (%)**	**Resin yield (kg/ha)**
AZ-1	20.3 a*	14.3 a	5.5 cd	789 ab	9.1 a	1158 a
AZ-2	19.4 ab	13.2 ab	6.2 abcd	771 ab	9.3 a	1115 ab
AZ-3	19.0 ab	12.2 ab	5.3 d	622 b	8.4 a	906 abc
AZ-5	18.7 ab	13.9 ab	7.3 ab	966 a	7.9 a	1135 ab
AZ-6	20.1 ab	14.6 a	6.1 bcd	855 ab	8.3 a	1049 ab
N 565	13.9 b	8.9 b	7.5 a	675 ab	8.3 a	727 bc
11591	15.0 ab	9.4 ab	6.9 abc	618 b	5.5 b	497 c
LSD$_{(0.05)}$	**7.4**	**5.4**	**1.4**	**331**	**2.2**	**418**

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

3.4.4 Rubber production

At 17 and 32 months, rubber content and yields showed significant differences among lines (Table 3.3 and 3.4). At 17 months, rubber content of all new lines except AZ-3 was not significantly different from the controls. AZ-5 had the highest rubber content of 8.4 % and it was significantly higher than AZ-3 (6.9 %). In general AZ-5 and AZ-6 (8.2 %) tended to have higher rubber contents than AZ-1 (7.4 %), AZ-2 (7.4 %) and AZ-3 (6.9 %).

In the first harvest, rubber yields of improved lines were not significantly different from one another but the rubber yield of AZ-1 (611 kg/ha) was significantly higher than N 565 (379 kg/ha) and 11591 (383 kg/ha). Yield increase of line AZ-1 was in the range of 59 % to 61 % compared to N 565 and 11591 (Figure 3.7). Rubber yield of AZ-2 (567 kg/ha) and AZ-5 (565 kg/ha) had 47% to 50% yield increase over the controls; however, these differences were not significant.

Rubber content at 32 months too showed significant differences among lines. At this age, rubber contents of AZ-5 (7.3 %) and AZ-2 (6.2 %) were not significantly different from both N 565 (7.5 %) and 11591 (6.9 %). Rubber content of 6.1 % for AZ-6 was significantly lower than N 565 but not 11591. Both AZ-1 (5.5 %) and AZ-3 (5.3 %) had significantly lower rubber content than the controls.

At the second harvest, rubber yield of AZ-5 (966 kg/ha) was significantly higher than 11591 (618 kg/ha) but not N 565 (675 kg/ha). The yield increase of AZ-5 over the controls was in the range of 43% to 56% (Figure 3.8). Yields of AZ-1 (789 kg/ha), AZ-2 (771 kg/ha) and AZ-6 (855 kg/ha) tended to be higher than the controls but the differences were not significant.

Rubber content of all lines decreased from harvest 1 to 2 (Figure 3.9). This reduction was higher for new lines and it ranged from 13 to 26 %. The decrease was smaller for the older lines, N 565 and 11591 (7 to 8 %). All lines increased rubber yield from harvest 1 to 2 due to increase in dry matter production (Figure 3.10). AZ-5 and AZ-6 produced 70 to 71 % increase in rubber yield while AZ-1, AZ-2 and AZ-3 had only 29 to 36 % increase in yield. AZ-5 had the highest or tended to have the highest rubber bearing plants among new lines for both harvests (Figure 3.9). The combined effect of high rubber content and, moderate to high dry matter yield of AZ-5 resulted in or tended to result in increased rubber yields at both harvests compared to controls (Figure 3.8).

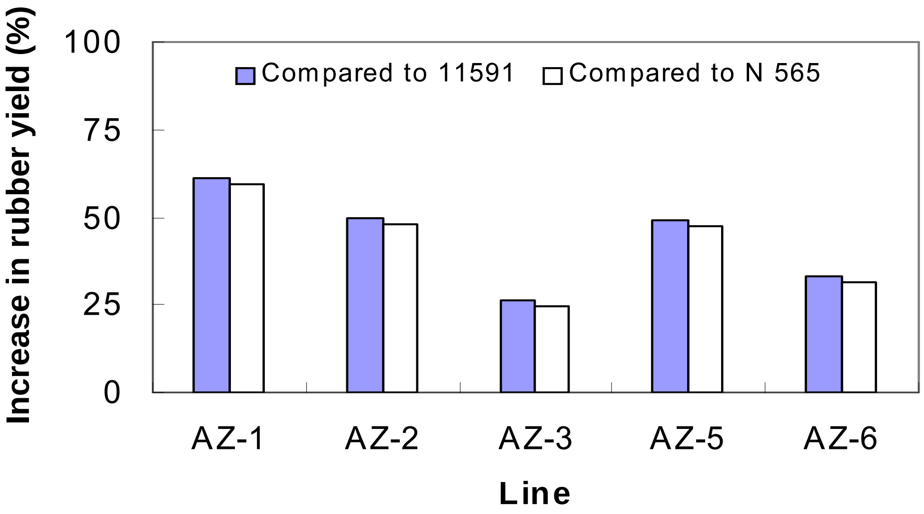


Figure 3.7. Comparative rubber yield increase of new lines over the controls at 17 months of age

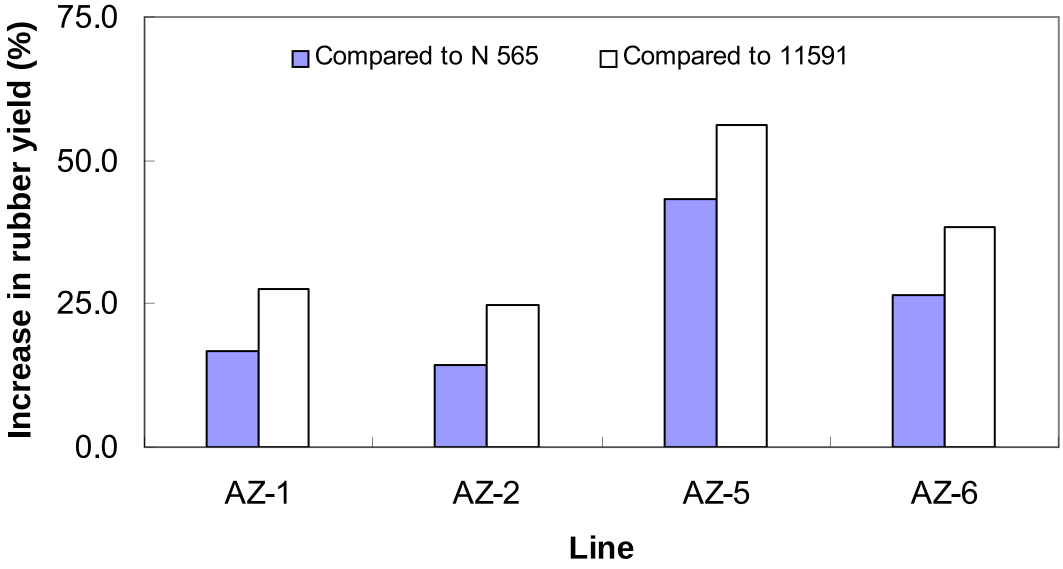


Figure 3.8 Comparative rubber yield increase of new lines over the controls at 32 months of age

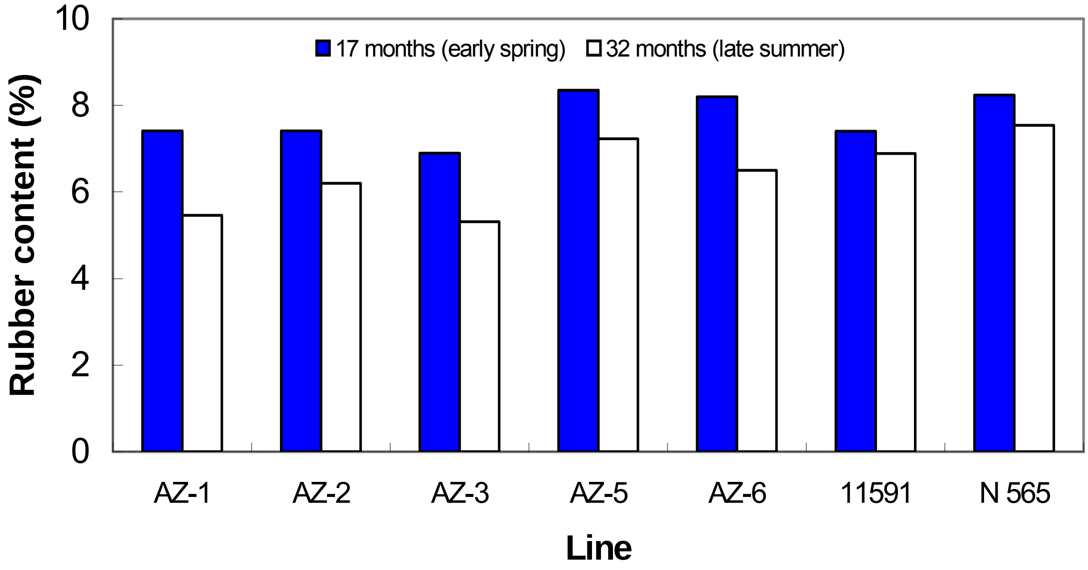


Figure 3.9 Rubber content of guayule lines in spring (17 months) and summer (32 months) at Gatton

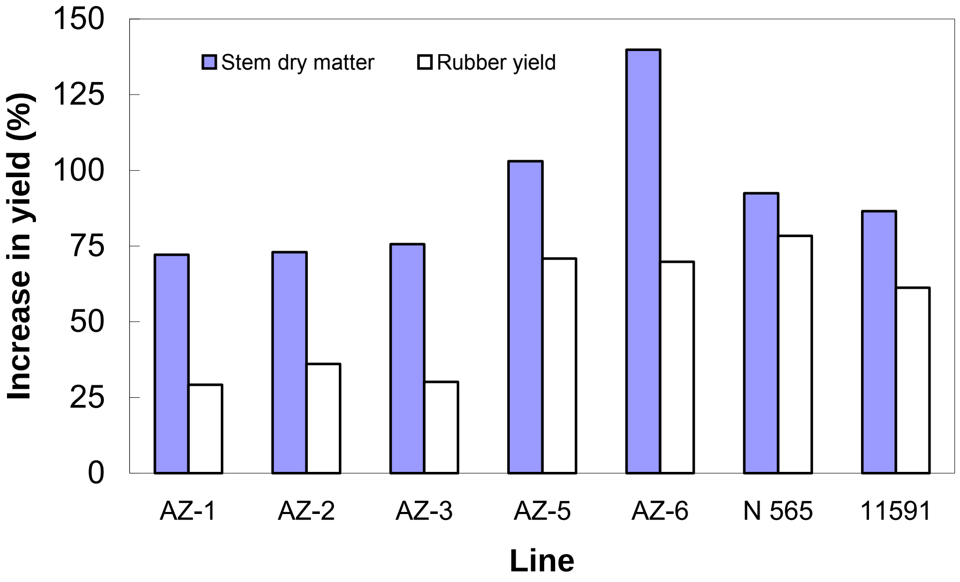


Figure 3.10 Increase in rubber and stem dry matter yields from 17 months to 32 months

3.4.5 Resin production

At the first harvest, resin contents of AZ1 (8.6 %), AZ-2 (8.7 %) and AZ-3 (8.7 %) were not significantly different from N 565 (9.4 %) (Table 3.3). Resin contents of AZ-5 (8.0 %) and AZ-6 (7.7 %) were significantly lower than N 565 but not with 11591 (6.5 %).

At this age, a resin yield of 725 kg/ha for AZ-1 was significantly higher tan N 565 (433 %) and 11591 (331 %). The yield increased over the controls was 119 % and 67% respectively (Figure 3.11). Resin yields of 663 kg/ha for AZ-2 and 604 kg/ha for AZ-3 were significantly higher than 11591, but not for N 565.

At the second harvest, rubber contents of all lines were lower than the first harvest. However, resin contents of all new lines to remained constant or tended to increase slightly (Figure 3.12). There were no significant differences among lines except that 11591 was lower than all other lines for resin content. Resin contents of N 565 and 11591 dropped by about 1 %.

Resin yields of all new lines were not significantly different from one another. Resin yield of 1158 kg/ha for AZ-1 was significantly higher than N 565 (727 kg/ha) and 11591 (497 kg/ha). All other new lines had significantly higher resin yield than 11591 but not N 565 (Figure 3.13).

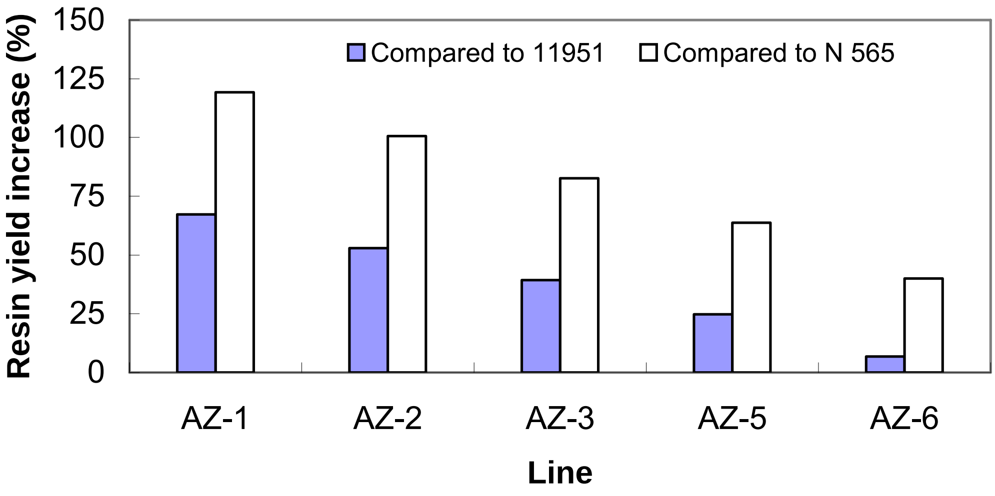


Figure 3.11 Comparative resin yield increase of new lines over the controls at 17 months

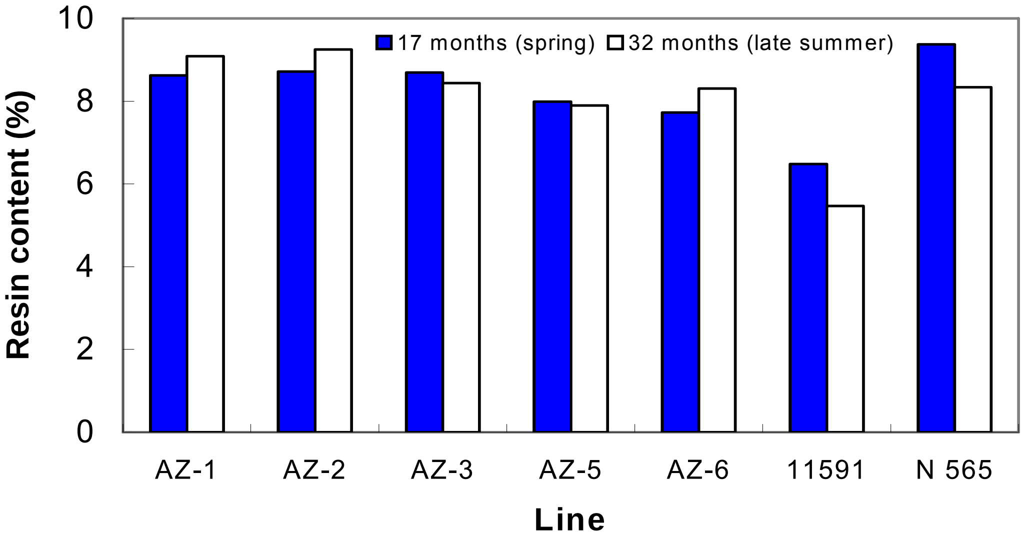


Figure 3.12 Resin contents of different lines at two harvests at 17 and 32 months

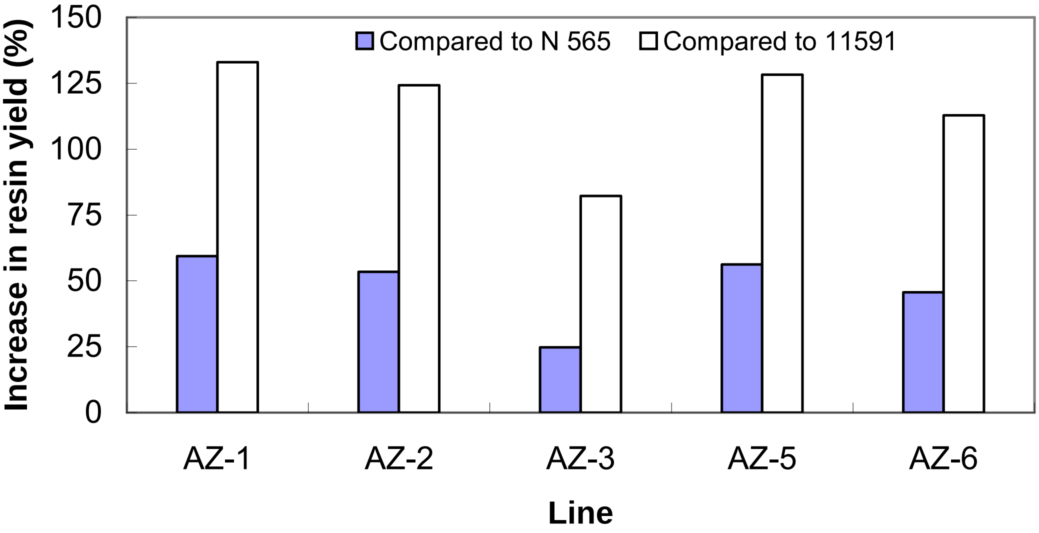


Figure 3.13 Comparative resin yield increase of new lines over the controls at 32 months

3.4.6 Pest and disease incidence

Young guayule seedlings in the nursery were affected by aphids but had relatively minor effect. Two root diseases caused death of some seedling while they were in the field. Initially when plants were about six months old, four dead plants were discovered in four different places in the trial area. The causal organism was identified as *Sclerotium rolfsii*. These were found during summer 2001 when soil moisture was optimum for plant growth.

A serious disease outbreak occurred when plants were 32 months. This outbreak started following heavy summer rainfall that inundated part of the field. Rainfall of 133mm received within 8 days with 105 mm rain in two consecutive days. The mean minimum and maximum temperature during the period was 22°C and 31°C respectively. Water logging lasted for about three days due to changed drainage conditions in the field. A week after this, one third of the plants in the trial were dead (Plate 3.2). The causal organism was later identified as *Rhizoctonia solani*. This event resulted in the second harvest occurred in February 2004 rather than in September as originally planned.

Plate 3.2 Plants affected by root rot caused by *Rhizoctonia solani* following heavy rains during summer 2004

3.5 Results – Chinchilla

3.5.1 Seedling establishment

The survival rate of transplants was over 86% for all lines except for line 11591 which had a survival rate of 77% (Table 3.5).

Table 3.5 Seedling establishment four weeks after transplanting

Line	**Mean Plant Establishment (%)**
AZ-1	95.0 ab*
AZ-2	96.5 a
AZ-3	92.0 ab
AZ-4	94.0 ab
AZ-5	92.0 ab
AZ-6	86.0 ab
N 565	86.5 ab
11591	77.5 b

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

3.5.2 Plant growth and variability

All new lines had comparatively faster growth rate than the controls (Figure 3.14 and 3.15). Of six new lines, AZ-1, AZ-2 and AZ-3 maintained the greatest height and width through out the study period (Plate 3.2). These three lines also had comparatively high uniform plant growth compared to other new lines.

AZ-4, AZ-5 and AZ-6 had two distinctly different types of plants, large and small. Both AZ-4 and AZ-5 had a mean of 14% to 15% large while AZ-6 had about 31% large plants (Figure 3.16). The variability of these lines (AZ-5 and AZ-6) was much higher compared to plant variability at Gatton.

Plate 3.2 Sixteen months old guayule plants at Chinchilla

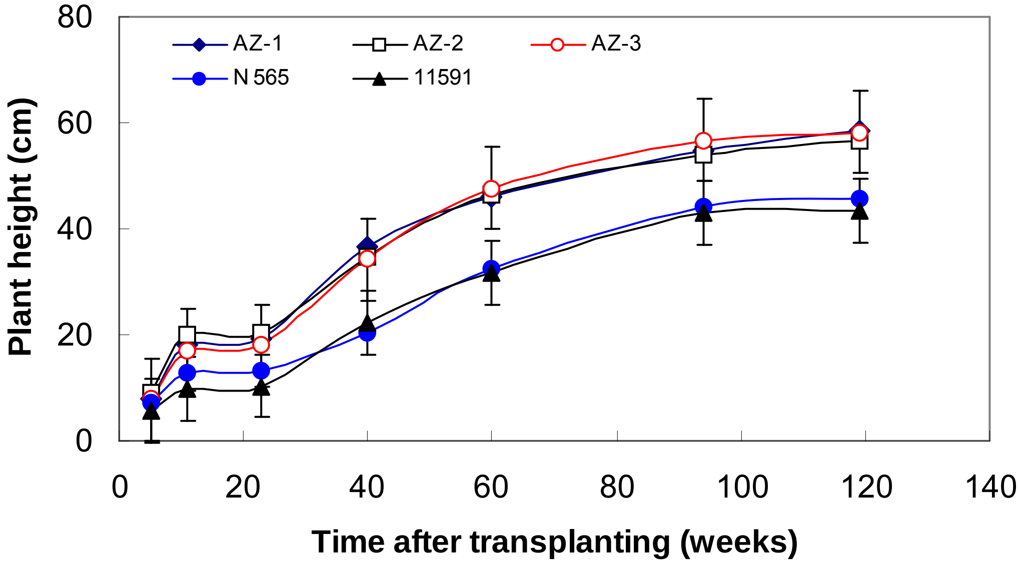


Figure 3.14 Plant height of selected guayule lines from transplanting to age 33 months at Chinchilla

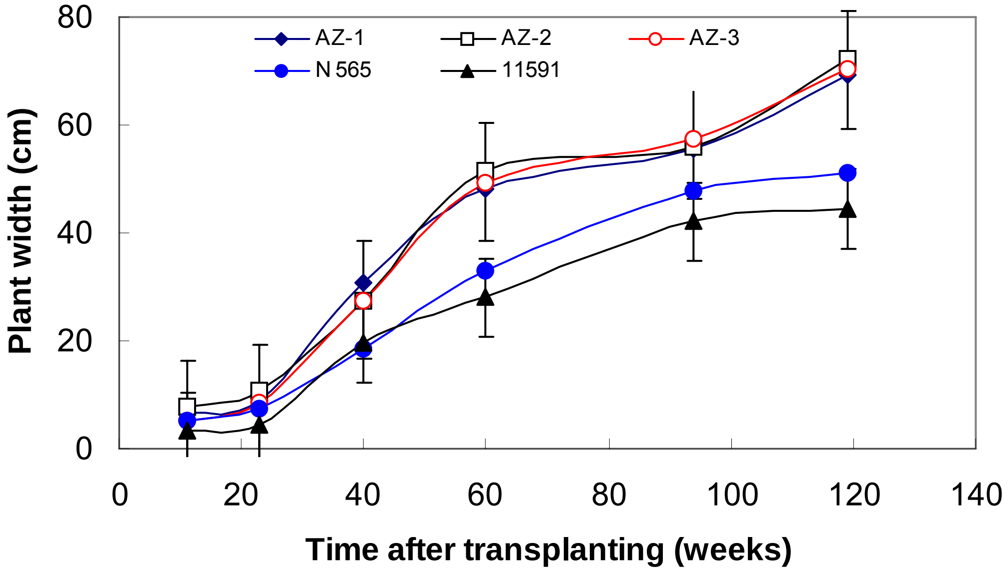


Figure 3.15 Plant width of selected guayule lines from transplanting to age 33 months at Chinchilla

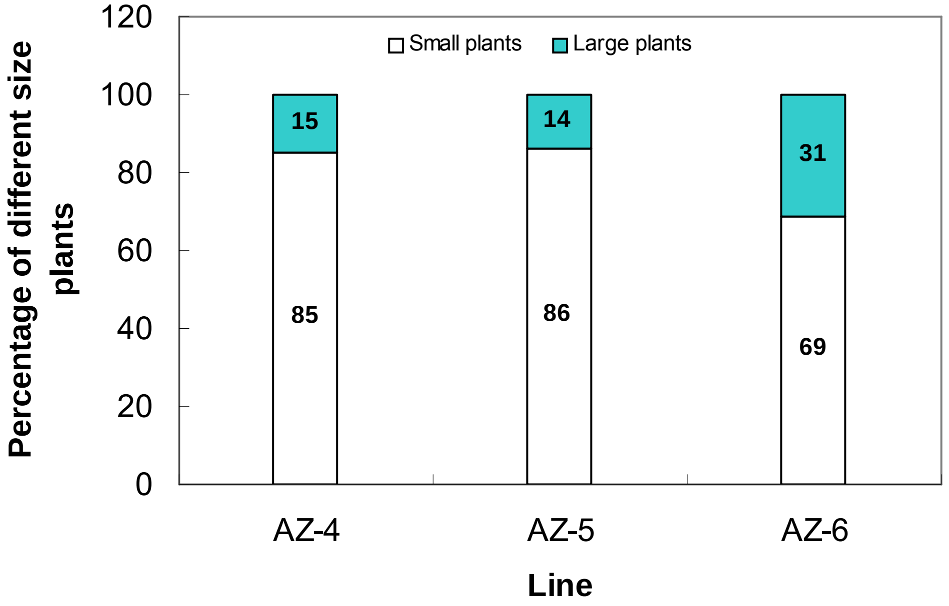


Figure 3.16 Percentage of large and small sized plants of three guayule lines, AZ-4, AZ-5 and AZ-6

3.5.3 Biomass production

Top growth (Stem, branch and leaf)

Stem, branch and leaf dry matter production at 33 months showed significant differences among lines (Tables 3.6). All new lines produced significantly higher dry matter yields than N 565 (6.2 t/ha) and 11591 (5.5 t/ha). Out of the six new lines, AZ-1 (13,7 t/ha), AZ-2 (14.1 t/ha), AZ-3

(12.3 t/ha) and AZ-6 (12.0 t/ha) had the highest dry matter yields. AZ-4 (10.7 t/ha) and AZ-5 (9.5 t/ha) produced the lowest dry matter among the new lines.

Stem and branch

Stem and branch dry matter production showed similar trends as for stem, branch and leaf dry matter (Table 3.6). All new lines, AZ-1 (11.6 t/ha), AZ-2 (11.7 t/ha), AZ-3 (10.6 t/ha), AZ-4 (9.1 t/ha), AZ-5 (8.1 t/ha) and AZ-6 (9.8 t/ha) produced significantly higher stem and branch dry matter yields than N 565 (4.8 t/ha) and 11591 (4.4 t/ha). The increase in stem and branch dry matter yields of AZ-1 and AZ-2 over the controls ranged from 140% to 164% while AZ-3 had 119% to 138% yield increase (Figure 3.17). Yield increases of AZ-4, AZ-5 and AZ-6 ranged from 68% to 120%.

Root

Root dry matter yields too showed significant differences among lines (Table 3.6). However, only AZ-1 (4.3 t/ha) and AZ-2 (4.9 t/ha) had significantly higher root dry matter yields compared to N 565 (2.0 t/ha) and 11591 (1.7 t/ha). These two lines produced 114% to 186% more root dry matter than the controls (Figure 3.18). AZ-3 (2.9 t/ha) and AZ-6 (3.1 t/ha) tended to produce higher root dry matter yields than the controls, however, they were not significantly different. AZ-4 had the lowest root dry matter among new lines and not significantly different from the controls.

Table 3.6 Mean of dry matter production of guayule lines at the age of 33 months at Chinchilla

Line	**Dry matter yield (t/ha)**			
	Stem, branch and leaf	**Stem and branch**	**Root**	**Total plant**
AZ-1	13.7 ab*	11.6 a	4.3ab	18.0
AZ-2	14.1a	11.7 a	4.9a	19.0
AZ-3	12.3 ab	10.6 ab	2.9 b	15.1
AZ-4	10.7 b	9.1 b	1.9 b	12.6
AZ-5	9.5 bc	8.1 b	2.1 b	11.6
AZ-6	12.0 ab	9.8 ab	3.1 ab	15.1
N 565	6.2 c	4.8 c	2.0 b	8.3
11591	5.5 c	4.4 c	1.7 b	7.2
LSD$_{(0.05)}$	**3.2**	**2.6**	**1.7**	

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

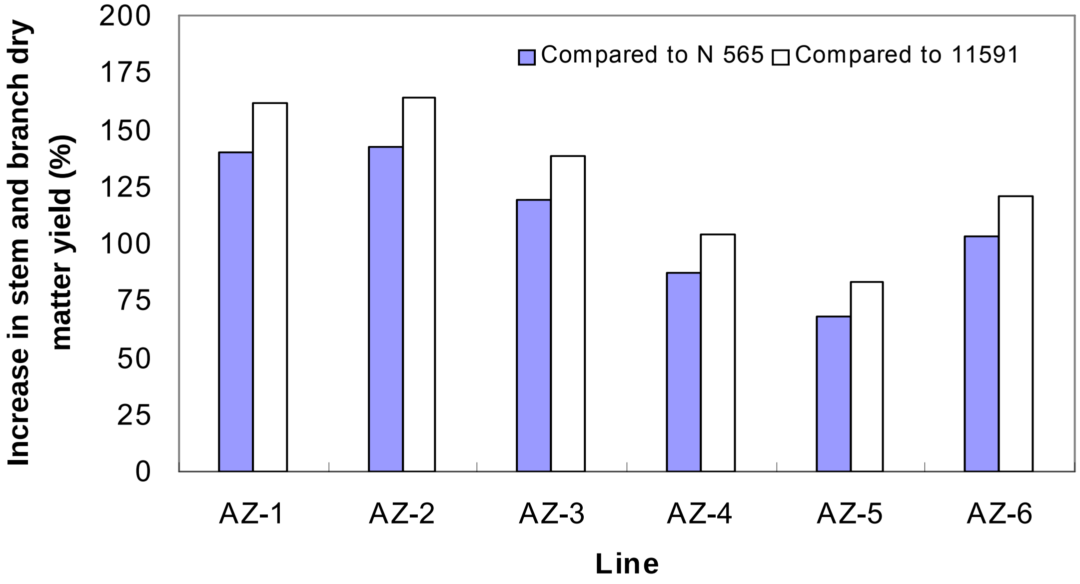


Figure 3.17 Comparative stem and branch dry matter yield increase of new lines over the controls at 33 months of age at Chinchilla

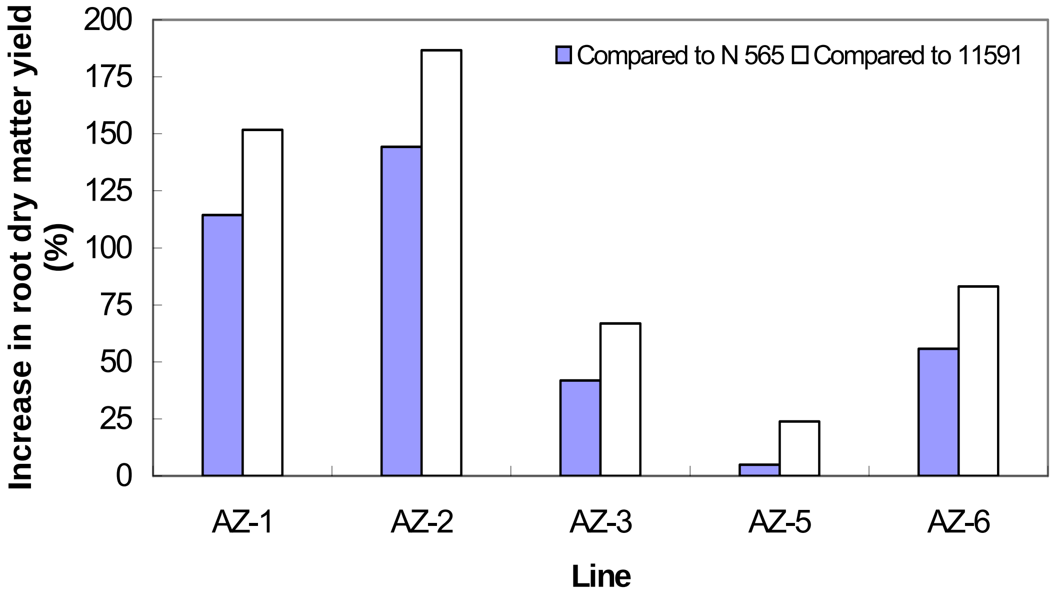


Figure 3.18 Comparative root dry matter yield increase of new lines over the controls at 33 months of age at Chinchilla

Variability in dry matter production

Stem and branch dry matter analysis of small and large plants showed significant differences for AZ-4, AZ-5 and AZ-6 (Table 3.7). However, root dry matter did not show significant differences between large and small plants. Analysis further showed that small plants of all three lines produced about 1/4 of the dry matter yield of larger plants.

Table 3.7 Stem and branch and, root dry matter yields of two types of plants in AZ-4, AZ-5 and AZ-6

	Dry matter (g/plant)					
	Stem and branch			**Root**		
Line	**AZ-4**	**AZ-5**	**AZ-6**	**AZ-4**	**AZ-5**	**AZ-6**
Large plants (g/plant)	1293	1148	1034	343	470	330
Small plants (g/plant)	331	309	281	57	52	90
P-value	0.003	0.015	0.000	0.145	0.058	0.070

3.5.4 Rubber production

Stem and branch

Stem and branch rubber contents showed significant differences among lines (Table 3.8). Stem and branch rubber contents of AZ-4 (8.3%), AZ-5 (8.0%) and AZ-6 (7.8%) were not significantly different from N 565 (7.9%) and 11591 (8.7%). Rubber contents of AZ-1 (6.2%), AZ-2 (6.6%) and AZ-3 (5.7%) were significantly lower than both the controls.

Stem and branch rubber yields of AZ-1 (717 kg/ha), AZ-2 (787 kg/ha) and AZ-6 (668 kg/ha) were significantly higher than N 565 (385 kg/ha) and 11591 (380 kg/ha) (Table 3.8). The yield increase of these three lines over the controls ranged from 73% to 107% (Figure 3.19). AZ-4 (626 kg/ha) produced a significantly higher rubber yield than 11591 but not for N 565. AZ-3 (608 kg/ha) and AZ-5 (574 kg/ha) also tended to produce increased rubber yields compared with the controls but the differences were not significant.

Root

In general, root rubber contents of all lines including controls were lower than stem and branch rubber content. Root rubber content of AZ-5 (3.7 %) was significantly lower than 11591 (5.7 %) but it was not significantly different from N 565 (5.0 %) (Table 3.8). All other new lines (ranged from 2.0 % to 3.1 %) had significantly lower rubber contents than the controls. Root rubber yield of new lines ranged from 55 t/ha to 111 kg/ha. These yield data were not significantly different from N 565 (102 kg/ha) and 11591 (93 kg/ha).

Table 3.8 Rubber contents and yield of new improved guayule genotypes compared with 11591 and N 565 at 33 months of age at Chinchilla

Line	**Rubber Content (%)**		**Rubber yield (kg/ha)**		
	Stem and branch	**Root**	**Stem and branch**	**Root**	**Total**
AZ-1	6.2 b*	2.3 cd	717 a	97	816
AZ-2	6.6 b	2.3 cd	787 a	111	909
AZ-3	5.7 b	1.9 d	608 abc	55	680
AZ-4	8.3 a	3.1 cd	626 ab	63	705
AZ-5	8.0 a	3.7 bc	574 abc	77	644
AZ-6	7.8 a	2.1 cd	668 a	66	731
N 565	7.9 a	5.0 ab	385 bc	102	480
11591	8.7 a	5.7 a	380 c	93	481
LSD$_{(0.05)}$	**1.2**	**1.6**	**244**	**ns**	

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

ns Non significant at 0.05 probability

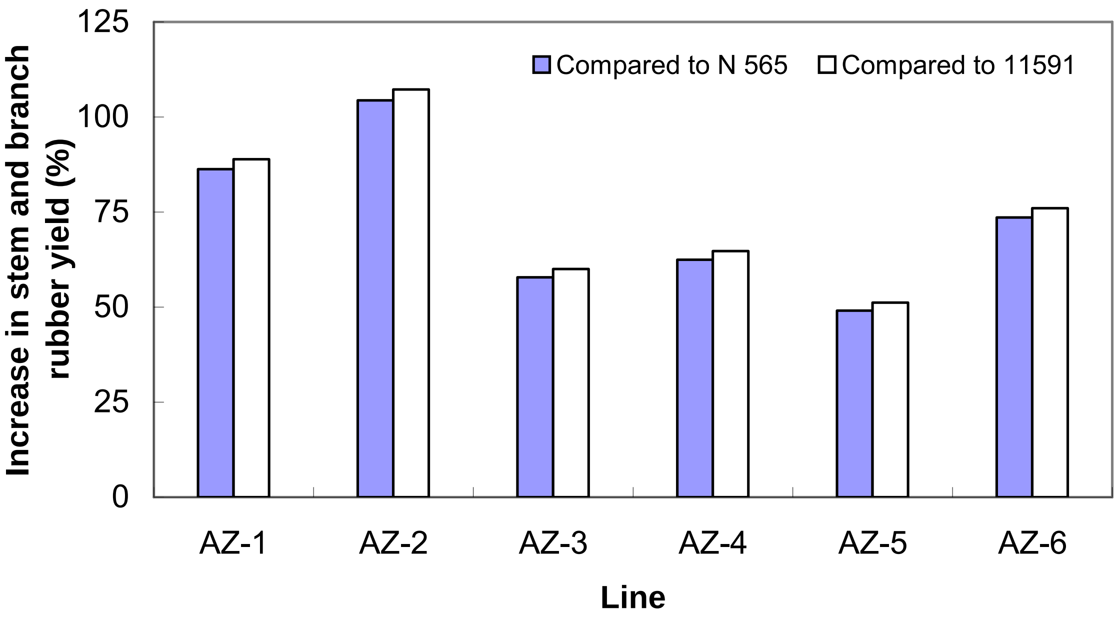


Figure 3.19 Comparative, stem and branch rubber yield increase of new lines over the controls at 33 months at Chinchilla.

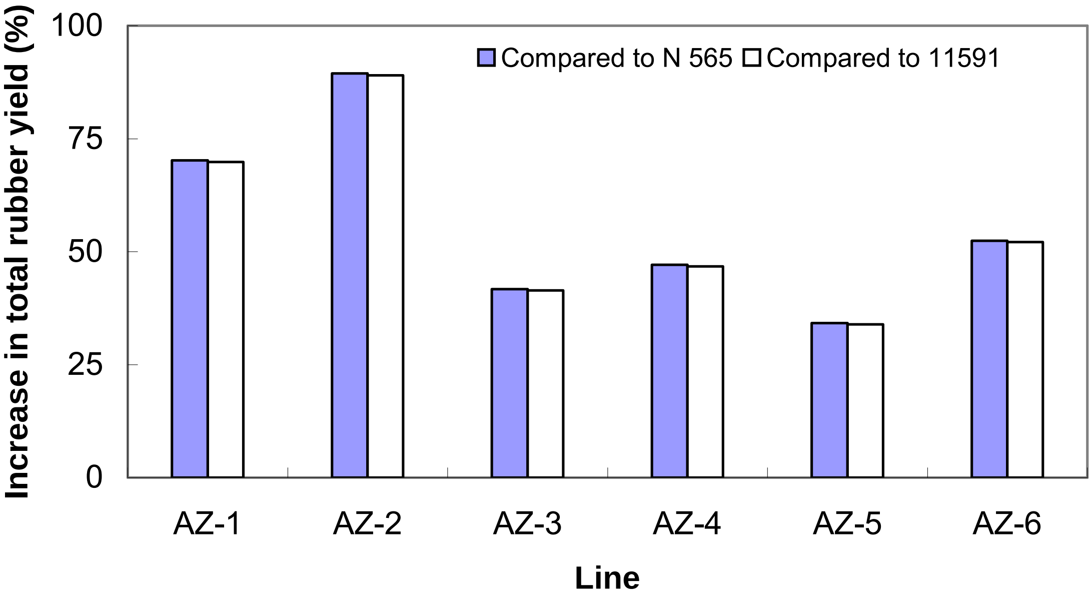


Figure 3.20 Comparative total plant (including roots) rubber yield increase of new lines over the controls at 33 months at Chinchilla.

Variability in rubber production

Stem and branch rubber contents and yields of the two plant types (small and large) in AZ-4, AZ-5 and AZ-6 also showed significant differences (Tables 3.9). Rubber contents of small plants of AZ-4 (8.8%), AZ-5 (8.5%) and AZ-6 (9.1%) were significantly higher than for large plants (4.9%, 4.9% and 5.1% respectively).

Table 3.9 Mean of rubber contents of stem and branch and, root of small and large plants of lines AZ-4, AZ-5 and AZ-6 at 33 months of age at Chinchilla

	Rubber content (%)			**Rubber yield (g/plant)**		
Line	**AZ-4**	**AZ-5**	**AZ-6**	**AZ-4**	**AZ-5**	**AZ-6**
Large plants	4.9	5.0	5.1	63	57	53
Small plants	8.8	8.5	9.1	29	26	26
P- value	0.001	0.000	0.002	0.016	0.057	0.122

Relationship between stem and branch rubber content and dry matter yield

A strong negative correlation with an R^2 of 0.91 was obtained between rubber content and stem and branch dry matter yield (Figure 3.21). Data grouped into three areas: the lower end of the curve represents the plants with high dry matter and low rubber contents (lines AZ-4, AZ-5 and AZ-6), the upper end of the curve stand for the small plants of AZ-4, AZ-5, AZ-6 as well as N 565 and 11591 and the middle data set represent lines AZ-1, AZ-2 and AZ-3. This indicates that AZ-1, AZ-2 and AZ-3 combine features of the genotypes at the two extremes.

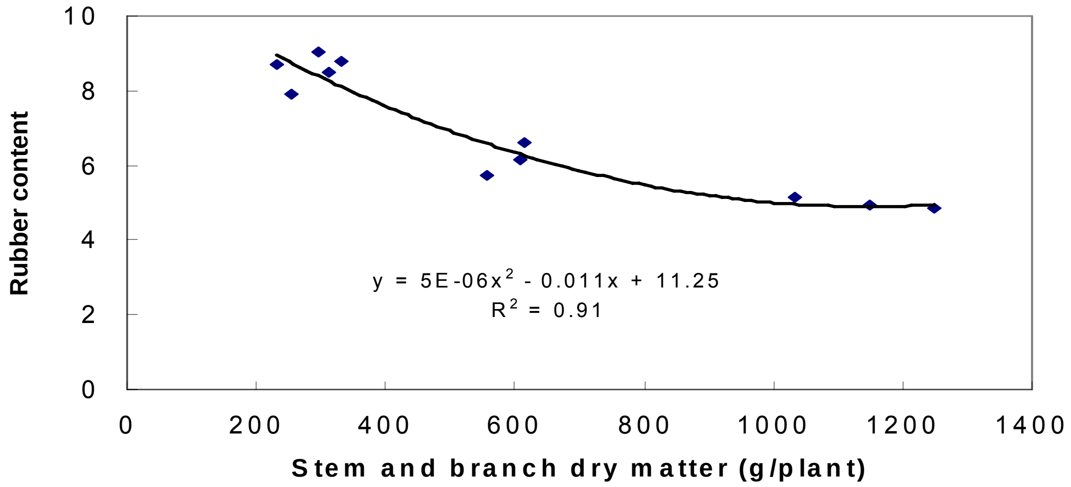


Figure 3.21 Relationship between biomass production and rubber content of different guayule lines and plant types at Chinchilla.

3.5.5 Resin content and yield

Stem and branch

Stem and branch resin content of all new lines (10.8 - 12.7 %) were significantly higher than N 565 (7.5 %) and 11591 (7.7 %) (Table 3.10). Resin yields too showed significant differences among lines. All new lines had significantly higher resin yields than the controls. Out of these six new lines, AZ-1 (1318 kg/ha), AZ-2 (1476 kg/ha). AZ-3 (1286 kg/ha) and AZ-6 (1104 kg/ha) produced the highest resin yields. The resin yield increases in these lines ranged from 202% to 337% (Figure 3.22). AZ-4 (970 kg/ha) and AZ-5 (922 kg/ha) also produced significantly higher resin yields than the controls. The yield increases of these two lines were between 152% to 187%.

Table 3.10 Resin contents and yield of new improved guayule genotypes compared with 11591 and N 565 at 33 months of age at Chinchilla

Line	**Resin Content (%)**		**Resin yield (kg/ha)**		
	Stem and branch	**Root**	**Stem and branch**	**Root**	**Total**
AZ-1	11.3 ab*	9.6 ab	1318 ab	414 ab	1732
AZ-2	12.7a	9.8 a	1476 a	486 a	1962
AZ-3	12.2 ab	10.0 a	1286 abc	285 bc	1571
AZ-4	10.8 b	7.6 bcd	970 cd	146 c	1116
AZ-5	11.1 ab	8.3 bcd	922 d	174 c	1096
AZ-6	11.5 ab	8.9 abc	1104 bcd	277 bc	1382
N 565	7.5 c	7.7 cd	365 e	158 c	522
11591	7.7 c	7.2 d	338 e	122 c	460
$LSD_{(0.05)}$	**1.8**	**1.5**	**342**	**193**	

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

Root

Root resin contents also showed significant differences among lines. Roots of AZ-1 (9.6 %), AZ-2 (9.8 %) and AZ-3 (10.0 %) produced significantly higher resin contents than N 565 (7.7 %) an 11591 (7.2 %). Resin content of AZ-6 (8.9 %) was significantly higher than 11591 but not for N 565.

Root resin yields of AZ-1 (414 kg/ha) and AZ-2 (486 kg/ha) were significantly higher than N 565 (158 kg/ha) and 11591 (122 kg/ha). Those two lines had 162 % to 298 % increase in root rubber yields (Figure 3.23). Other new lines except AZ-4 also had higher resin yield than both the controls but differences were not significant.

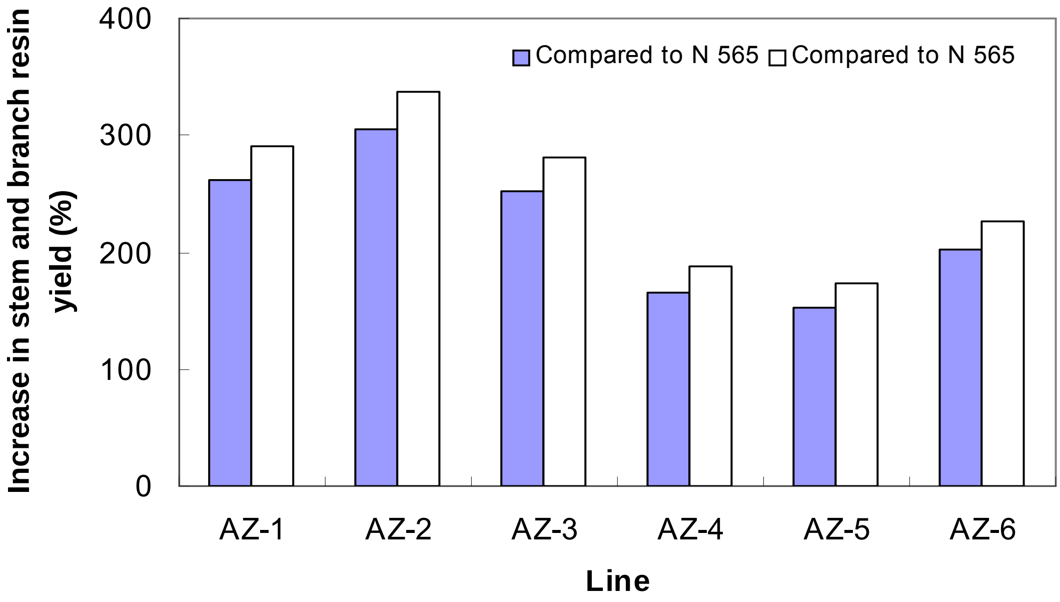


Figure 3.22 Comparative stem and branch resin yield increase of new lines over the controls at 33 months of age at Chinchilla

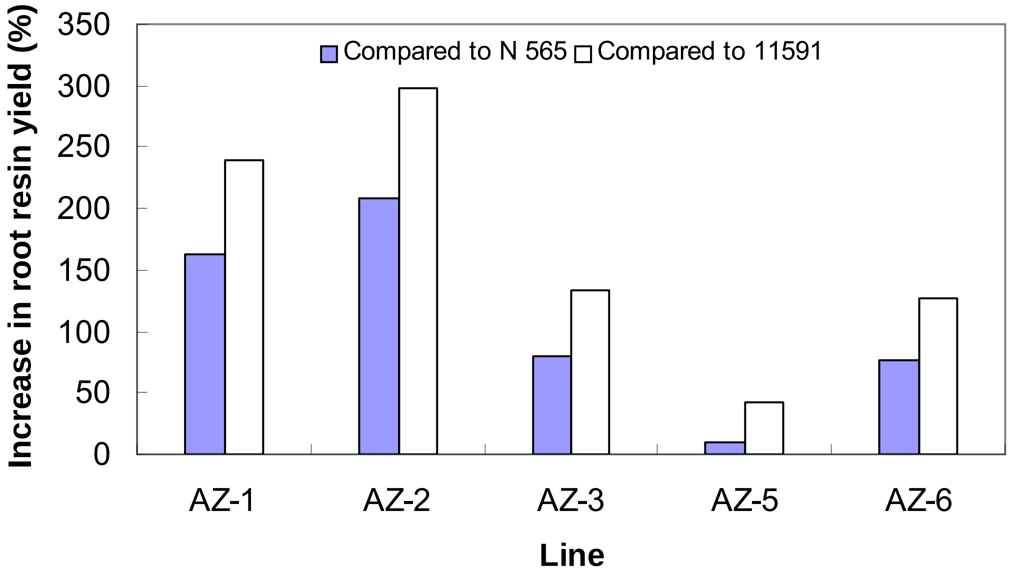


Figure 3.23 Comparative root resin yield increase of new lines over the controls at 33 months of age at Chinchilla

Variability in resin production

Unlike rubber contents, stem and branch resin contents of the two plant types (small and large) for lines AZ-4, AZ-5 and AZ-6 showed no significant differences (Tables 3.11). Both large and small sized plants had similar resin contents. However, resin yields between these two plant types had significant differences due to differences in dry matter yields.

Table 3.11 Stem and branch resin contents and yields of small and large plants of lines AZ-4, AZ-5 and AZ-6 at 33 months of age at Chinchilla

	Resin content (%)			**Resin yield (g/plant)**		
	AZ-4	**AZ-5**	**AZ-6**	**AZ-4**	**AZ-5**	**AZ-6**
Large plants	10.7	10.9	11.2	137	126	116
Small plants	10.9	11.1	11.6	36	34	32
P-value	0.848	0.747	0.648	0.003	0.012	0.000

3.5.6 Pest and disease incidences

Only minor pest problems were observed during the study period. Aphid attack was observed at the initial stage of seedling growth when seedlings were about 4 weeks old. Grasshoppers and cockatoos attacked young seedlings when they were about one to two months old. Thereafter, no pest and disease incidence was noticed during the study period.

3.6 Across site analysis

Three parameters of stem and branch dry matter, rubber content and rubber yield for the seven common lines (AZ-1, AZ-2, AZ-3, AZ-5, AZ-6, N 565 and 11591) followed similar trends at both sites (Figure 3.24, 3.25 and 3.26). All three parameters had significant differences between two sites (Table 3.12, 3.13 and 3.14). Mean stem and branch dry matter production for seven common guayule lines at Gatton (12.3 t/ha) was significantly higher than at Chinchilla (9.2 t/ha). Therefore, Chinchilla plants produced only about 74% of the stem and branch dry matter of Gatton plants.

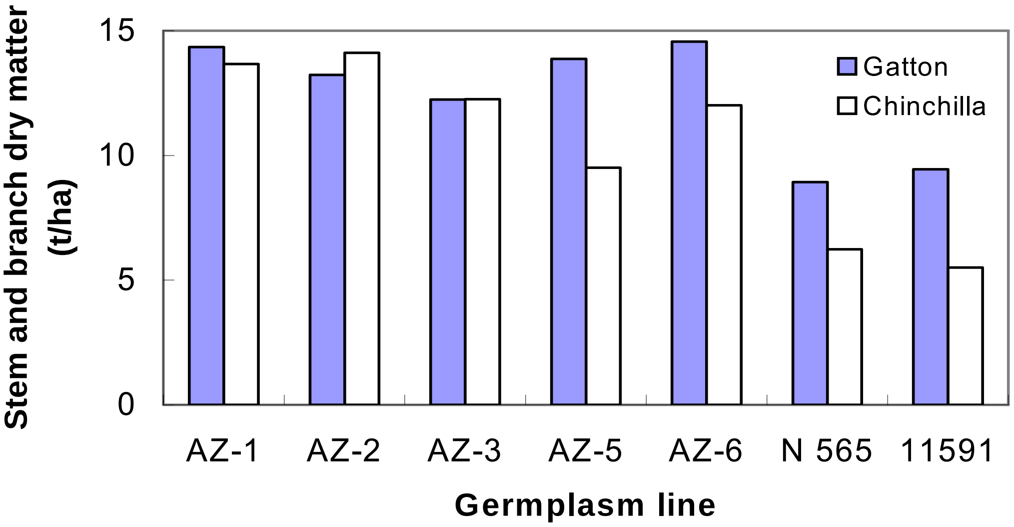


Figure 3.24 Stem and branch dry matter yield of guayule genotypes at two environments (Gatton and Chinchilla)

Table 3.12 Analysis of variance for stem and branch dry matter production between two sites

Source	DF	P-value
Site	1	0.000
Block(Site)	5	0.521
Line	6	0.000
Site x Line	6	0.423

Rubber content of all genotypes followed a similar trend at both environments at Gatton and Chinchilla (Figure 3.25). Cross-site analysis showed that rubber content of Gatton plants (6.4%) was significantly lower than that of Chinchilla (7.3%). Despite lower rubber content, Gatton plants produced a mean rubber yield of 748 kg/ha which was significantly higher than the mean rubber yield at Chinchilla (589 kg/ha).

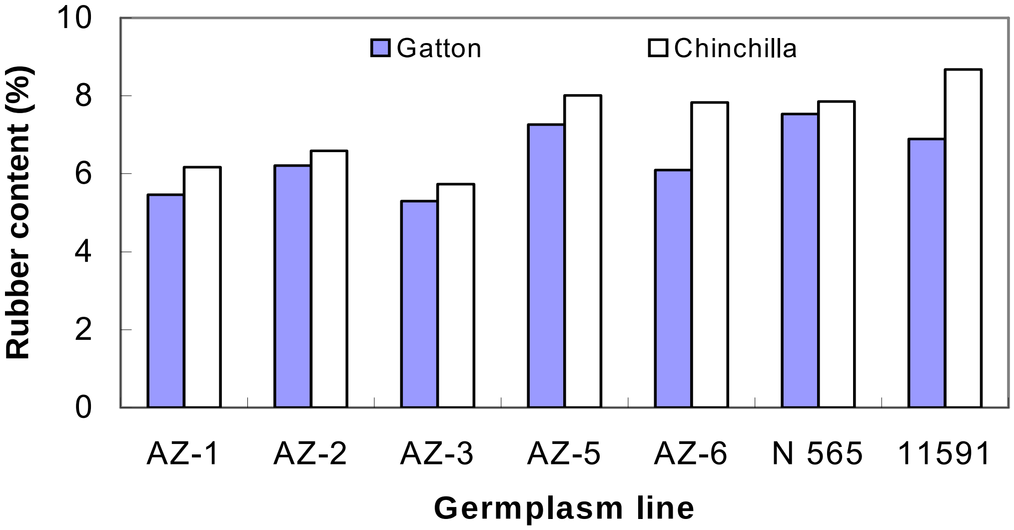


Figure 3.25 Rubber contents of guayule genotypes at two environments

Table 3.13 Analysis of variance for stem and branch rubber content between two sites

Source	DF	P-value
Site	1	0.001
Block(Site)	5	0.925
Line	6	0.000
Site x Line	6	0.411

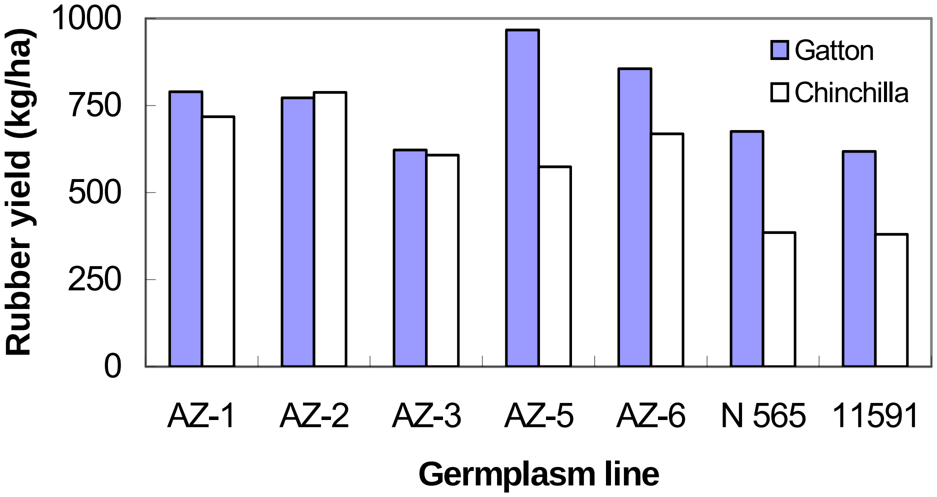


Figure 3.26 Rubber yield of guayule genotypes at two environments

Table 3.14 Analysis of variance for stem and branch rubber yield between two sites

Source	DF	P-value
Site	1	0.002
Block(Site)	5	0.592
Line	6	0.007
Site x Line	6	0.191

3.7 Discussion

3.7.1 Establishment, plant growth and biomass production

Establishment of transplants was well over 86% for most lines at both locations except two occasions, AZ-5 at Gatton and 11591 at Chinchilla. Comparatively lower establishment of AZ-5 at Gatton could mainly be due to effect of tannings released by the potting media used for nursery planting. Because 80% of potting media used in second and third months constituted of pine bark and saw dust. Weak growth and death of seedlings of some lines with maximum number for AZ-5 was observed at the end of the third month.

Bacterial blight cause death of some seedlings raised for Chinchilla trial. 11591 seedlings were the most affected by this disease. The lower establishment by 11591 could be due to this condition.

Plant growth (height and width) results obtained from both environments at Gatton and Chinchilla indicated that all new lines were better than previously developed cultivars. Of the new lines, AZ-1, AZ-2 and AZ-3 performed better with early vigour, fast and uniform growth and increased dry matter. Of these three, AZ-2 and AZ-3 produced the most uniform plant canopies throughout the study period at both locations indicating high levels of heritability. AZ-1 was comparatively less uniform in plant canopy development than AZ-2 and AZ-3. These three lines had the best plant growth and produced the highest dry matter at Chinchilla for the study period of 33 months. AZ-1, AZ-2 and AZ-3 also produced the best results at Gatton during first two years for the same parameters. However, in the third year at Gatton, AZ-5 and AZ-6 also produced increased dry matter comparable with AZ-1, AZ-2 and AZ-3.

One of the criteria in the selection of these new lines was their early vigorous growth (Ray *et al.* 1999). Thus, results indicate that heritability of plant growth was highest for AZ-2 and AZ-3. AZ-1 could be ranked as the third among new lines. This early fast growth could improve the commercial potential of guayule in two ways. As indicated by Ray *et al.* (1999), it could decrease time to initial harvest from three to two years. Vigorous growth also could improve the field establishment of guayule by making seedlings more competitive with weeds because guayule, in general, is slow growing and not a good competitor (Milthorpe, 1984).

AZ-4, AZ-5 and AZ-6 produce moderate dry matter yields at Chinchilla. AZ-5 and AZ-6 produced moderate dry matter yield in the second year and high dry matter yield in the third year at Gatton and dry matter yields at this stage were comparable with AZ-1, AZ-2 and AZ-3. However, plant variability of AZ-4, AZ-5 and AZ-6 was always high compared to AZ-1, AZ-2 and AZ-3. Further, plant variability was much higher at Chinchilla than at Gatton.

High heritability could mainly be due to high levels of apomixis in AZ-2 and AZ-3. Highly variable plant growth by AZ-4, AZ-5 and AZ-6 indicates reproduction by both modes of apomixis and sexual. Distinctly different plant growth (Large and small plant) could be due to segregation of characters due to sexual reproduction.

The differential results for two sites could either be due to environmental effect or due to genotype effect or combined effect of both. Because seed obtained for these two trials received at two different occasions. Dierig *et al.* (2001) found that heritability decrease with the age of plants where it remains high for many traits (height and latex content) during first two years and effectively diminishes thereafter. These results indicate that crop improvement in guayule is relatively underdeveloped and there is potentially much scope for future development.
Stem dry matter production of 11591 and N 565 in studies conducted in Australia at Kingaroy (Ferraris, 1993) and Hilston (Milthorpe *et al*., 1994) was reported to be less than 8 t/ha even though the crop was three years old. In another Australian study, Downes and Tonnett (1985) reported a maximum dry matter yield of 316 g/plant at Narrabri and 348 g/plant at Canberra. These values can be extrapolated as 8.7 t/ha and 9.6 t/ha respectively at the recommended population of 27,500 plants per hectare. The same lines in the current study produced a comparable yield of about 9 t/ha of stem and branch dry matter for 32 months old plants at Gatton. However, they produced a half that yield at Chinchilla (4.8 t/ha for N 565 and 4.4 t/ha for 11591). These results indicate that higher dry matter yields for new lines compared with the older controls are most likely due to genetic improvement. Further, data show that environment at Gatton is more favorable for growth of guayule than the environment at Chinchilla.

3.7.2 Effect of environment and plant population on dry matter yield

Cross-site analysis for stem and branch dry matter yields showed that the Gatton site (12.3 t/ha) had more favourable conditions for growth of guayule than Chinchilla (9.2 t/ha). Soil could be the main factor influencing dry matter production. Because the soil at Gatton was high in clay content and able to hold more soil moisture than the Chinchilla soil, plant growth would be enhanced at the Gatton site. Higher summer maximum and lower winter minimum at Chinchilla compared to Gatton would also effect plant growth, higher temperature would increase moisture stress whereas lower temperature would induce growth rates.

Both the Chinchilla and Gatton trials used a plant population density of 19,048 plants per hectare with a 1.5 m row spacing. However, average plant width of the largest plants at Chinchilla was only about 72 cm. Therefore in terms of canopy covers about half of the row spacing was utilized. At Gatton, the average plant width of the largest plants was about 90 cm. Therefore, there is a potential to increase canopy cover and dry matter by using a closer row spacing and increasing plant population.

3.7.3 Rubber content and yield

Stem and branch

Rubber contents obtained from all guayule genotypes in both environments (in the second year at Gatton and in third year at Chinchilla) were higher than the in the USA by Ray *et al*. (1999). The only exception was AZ-1 which had a lower rubber content at Chinchilla (6.2 %) than in the USA (7.1 %).

In their third year at Gatton (32 months), rubber content of some of the lines was lower (AZ-1 of 5.5%, AZ-5 of 7.3 %, AZ-6 of 6.1 % and N 565 of 7.5 %) than the USA values (AZ-1 7.7 %, AZ-5 7.5 %, AZ-6 7.3 % and N 565 8.0 %). The lower rubber content at Gatton was not due to the environmental effect but it was seasonal effect. Because those plants were harvested during summer when plants were actively growing without accumulation more rubber (Hammond and Polhamus 1965; Ji *et al.* 1993), this diluted the existing rubber concentration in the plant.

In another US study by Dierig *et al.* (2001), rubber contents of two years old lines AZ-2 (5.5 %), AZ-5 (6.3 %) and AZ-6 (6.4 %) were lower than corresponding values obtained from the current study. In an Argentinean study (Coates *et al*., 2001), rubber content of four lines common to the present study showed contrasting results. In their study, AZ-3 (6.45%) had a similar rubber content to the controls (8.0% for 11591 and 7.0% for N565) and AZ-5 (5.3%) was less than the standard lines.

In other studies in Australia at Kingaroy and Hillston, high rubber contents of 11.9% and 13.0% for N 565 and 11591 after three years were reported (Milthorpe *et al.*, 1994; Ferraris, 1993). Downes and Tonnett (1985) reported concentration of 12% and 21% rubber. Comparatively higher rubber content in Australia compared with the U.S. would indicate genotype x environment interaction.

AZ-1 and AZ-2 had moderate rubber content throughout the study periods for two different environments. They also tended to produce high dry matter througout the study period for the two environments and therefore tend to produce high rubber yields. AZ-3 had a vigorous and uniform plant growth, however, it had the lowest rubber content irrespective of time and environment that resulted in lower rubber yield.

In the third year at Gatton AZ-5 and AZ-6 had high rubber yields due to the combined effect of high rubber content and dry matter. Rubber yield results support the suggestion by Ray *et al.* (1999) that some lines could be harvested in a shorter period, because AZ-1 and AZ-2 produced better yields when they were at their second year. However, results indicated that conditions should be favourable to harvest them in two years. In contrast, AZ-5 and AZ-6 produced better yields when they were three years old.

Roots

Root rubber contents for almost all lines was observed to be about third of stem and branch rubber. It was observed that roots harvested at Chinchilla had a higher proportion of larger roots than small fibrous roots. This could be the effect of environment especially soil type. Because the Chinchilla soil was a sandy loam with little clay content, this induced roots to grow deeper in response to soil moisture. A higher proportion of larger roots increased the wood to bark ratio and, in general, wood has a low rubber content than bark (Kuruvadi *et al.* 1997). Therefore, the low rubber content in roots could be due to high wood to bark ratio.

3.7.4 Seasonal effect on rubber content

All lines including the older lines had higher rubber content at 17 months of age when they were harvested in spring 2002 compared to 32 months (summer 2003). This decrease in rubber content can be explained by seasonal effects. Guayule, in general, follows a cyclic pattern of plant growth and rubber accumulation. The plant grows during the warmer months without accumulation much rubber. During the colder months, plants grow more slowly while accumulating rubber (Hammond and Polhamus 1965; Downes and Tonnett, 1985; Ji *et al.* 1993; Coates *et al*. 2001). Lower rubber content in the second harvest at Gatton compared to the first harvest was likely due to this phenomenon in which a plant grows without accumulating much rubber during warmer months. This suggests that spring would be the ideal time to harvest plants for highest rubber content and yields.

3.7.5 Resin content and yield

All new lines had comparatively high resin contents for both environmental conditions. In the second year at Gatton, resin content ranged from 7.7% to 8.7% while in the third year it varied from 7.9% to 9.3%. However, the Chinchilla plants had higher resin content than the Gatton plants ranging from 11.1% to 12.7% for stem and branch and 8.3 to 10.0% for roots. The results showed that differences in resin contents between lines were narrower than the rubber contents. Resin contents reported from a US study ranged from 8.6% to 10.0% (Ray *et al*. 1999) and these results are comparable with Gatton data.

Unlike rubber content, seasonal changes seem to have little or no effect on resin content. However, environmental conditions seem to have effect on resin contents. Chinchilla plants had more resin contents than Gatton plants. Comparatively higher maximum and lower minimum temperatures as well as sandy soil at Chinchilla could be favaourable for resin production. Root resin contents showed slightly lower value than stem and branch resin contents. It could be due to high wood to bark ratio in roots as described in section 3.7.3.

High resin contents combined with high dry matter yields resulted in high resin yields for all new lines both at Gatton and Chinchilla. AZ-1 and AZ-2 had highly consistent resin content and yield for different times of harvest and for different sites.

3.7.6 Pest and diseases

As reported by Milthorpe (1984) and Ferraris (1993), root disease is one of the most important biotic factors that could cause problems in commercial plantings of guayule. *Rhizoctonia solani* caused a serious problem in the current study due to problems in drainage following heavy rains. *Phytophthora* and *Rhizoctonia* spp. were reported to cause serious problems in New South Wales, Australia (Milthorpe, 1984). *Fusarium* spp caused severe problems at Kingaroy, Queensland (Ferraris, 1993). *Verticillium*, *Pythium*, *Phytophthora* and *Fusarium* spp have been reported to cause problems in the USA (Siddiqui *et al*. 1982; Stone and Fries 1986; Ykema and Stutz 1991). Spread of root diseases favoured by the combination of high temperature and poor drainage associated with high clay soils. Therefore, precautions should be taken to avoid or minimize the spread of diseases. Proper drainage is very important for guayule to avoid or minimize soil saturation. Also whenever possible, avoid use of high clay soils for growing guayule commercially especially within high rainfall areas.

A few minor pest problems occurred during the cause of the study. Aphids fed on young seedlings and cockatoos and galahs damaged young guayule plants. The same pests were reported to cause minor problems in New South Wales (Milthope, 1984).

4. Seed dormancy in guayule

4.1 Introduction

Seed dormancy is a main constraint to commercialisation of guayule because high levels of germinable seed are required for transplant or for direct seeding. The latter is needed to lower establishment cost. Two types of dormancy have been reported for freshly harvested guayule seed, a 6-12 months long seed coat dormancy and about a two months long endogenous dormancy (Benedict and Robinson 1946).

Light sensitivity is also an important issue with guayule seed (Benedict and Robinson 1946; Emparan and Tysdal 1957; Hammond 1959). Exposure to light during imbibition and early stages of germination increases the percent germination of guayule seed (Emparan and Tysdal 1957; Hammond 1959; Chandra 1991). Emparan and Tysdal (1957) and Hammond (1959) reported that exposure to light for 3 to 4 days during germination, plus oxidation by using 0.75% NaOCl, completely breaks the dormancy of freshly harvested guayule seed. Hammond (1959) further reported that gibberellins substitute for light by completely overcoming both embryo and inner seed coat dormancy. However, later studies by Naqvi and Hanson (1980) and Chandra and Bucks (1986) reported that gibberellic acid effectively replaced the light requirement but could not completely substitute for it. Therefore, researchers differed in the extent that gibberellic acid overcomes guayule seed dormancy.

Seed dormancy, a light requirement necessitating shallow planting, low seedling vigour and a slow growth rate, all affect establishment. Because establishment costs are high, this is a major barrier to commercialization of guayule. In particular knowledge on seed dormancy and its physiological causes was incomplete and past research was contradictory in some aspects. Therefore, the objectives of this study were to investigate seed dormancy with special attention to embryo dormancy and light requirement and hence fill the knowledge gap to facilitate improvements in establishment.

4.2 General materials and method

4.2.1 Seed collection and cleaning

Seed used in all germination tests was harvested from AZ-2, one of the promising lines in the germplasm evaluation trial established at Gatton. Seed was harvested on four occasions, November 2003 (late spring), February 2004 (late summer), April 2004 (mid autumn) and October 2004 (mid spring), when plants were two to three years of age. Mature seed heads were identified visually by the colour change from yellow to brown. These mature seed heads were harvested by manually stripping off them from the stalks. Harvested seeds were air-dried for a few days. Harvested material was then threshed manually to minimize seed damages. A round hole sieve of 1.18 mm diameter was used to remove particles smaller than the seed. A sieve size of 2.36mm was employed to separate unthreshed seed heads from the threshed seed. Unthreshed material was then rethreshed manually and processed as before. This procedure was carried out several times to maximize seed quantity. Later a laboratory seed blower was used to remove most of the empty and half-filled seed and, lightweight debris in the seed lot. The seeds were then separated manually from other particles of similar size using a magnifying lamp. Seed blower was used again to separate more empty and half-filled seed. The procedure produced 75 - 86 % filled seeds. Cleaned seed was stored at room temperature (monthly minimum and maximum temperature for during storage was between 31.4°C and 13.9°C except 10 months old seed used in section 4.3.3 that stored during winter with minimum temperature of 6.3°C) during the period of study.

4.2.2 Viability Test

Each clean seed lot was tested for viability before germination testing. Three samples each of 100 seeds were selected to test viability. They were soaked overnight in water between paper towels surrounded by cotton clothe. Then under the magnifying lamp, the seed coats were removed using a scalpel blade. The number of embryos obtained from 100 seeds was counted. These embryos were placed in a container with 1% tetrazolium solution and placed in an oven at 40°C for 3 hours. Finally viable embryos were counted based on pigmentation.

4.3 Germination tests

A series of germination tests were carried out in the Queensland Seed Technology Laboratory located on the Gatton Campus of the University of Queensland. Both coat intact seed and naked embryos (decoated seed) were used. Unless specified, a sample size of 50 randomly selected seeds was used and they were replicated three times. Germination tests were carried out in 10 cm diameter glass petri dishes with three layers of filter papers. Seed germination carried out under light was exposed to 8/16 hours of light and dark daily. Dark germination was conducted in a separate cabinet with no light and petri dishes were placed in empty metal biscuit containers (19 cm diameter and 6.5 cm height) and wrapped up with dark cotton towels to prevent light contamination. The temperature optimum for germination of guayule seed of 20°C (Cruz, 2003) was maintained for all tests. Germination tests were conducted for the period of 12 to 14 days.

Samples of 75 to 100 seeds were soaked overnight between paper towels surrounded by cotton cloth to obtain naked embryos (decoating). Under the magnifying lamp seed coats were carefully removed using a scalpel blade. A sample of 50 embryos was selected for testing germination. In some cases removal of seed coat (decoating) was undertaken after four hour soaking period. Seeds intended for use in dark germination were decoated with minimal light exposure (i.e. without using the light in the magnifying lamp). Soon after decoating, embryos were placed on moisten filter paper in petri dishes that were kept in dark container (empty metal biscuit container).

Guayule achene has two coats outer one is fruit coat (pericarp) and it is brittle and is easy to remove. Inner coat is the seed coat. The seed coat is thin and somewhat difficult to remove without damaging the embryo. Measures were taken to either remove this inner seed coat or to damage it by making a small cut at the cotyledon end to enable exchange of gases and water.

4.3.1 Effect of seed coat and light on germination

4.3.1.1 Introduction

Light is an extremely important environmental factor for overcoming dormancy in many small-seeded plant species (Bewley and Black, 1994). Almost all light requiring seeds have coat-imposed dormancy (Taiz and Zeiger, 2002). The response to light is species specific. Seeds of many species are affected by brief exposure to normal white light (few minutes or seconds) while others require intermittent illumination. Some species have a photoperiod response, requiring exposure to long days while others need short days (Bewley and Black, 1994; Taiz and Zeiger, 2002).

Seed coats in some plant species impose dormancy by various mechanisms. These involve prevention of water uptake, mechanical constraint to the radicle breaking through the seed coat and the action of inhibitory chemicals in the coat (ABA and phenolic compounds). The seed coat of some species is suspected of interference with gas exchange but this issue is still remains unresolved (Taiz and Zeiger, 2002).

Guayule is a small-seeded species that require light for breaking dormancy. It is also believed to have a coat-imposed dormancy (Benedict and Robinson, 1946). However, the mechanism or cause of seed dormancy is not well demonstrated. The objective of this experiment was to investigate the effect of seed coat and light on germination and to quantify their effects.

4.3.1.2 Materials and method

A series of germination tests were carried out using seeds harvested on three occasions from the same crop and cultivar (AZ-2); thus the seed is of different ages (from 19 days to 8 months). Germination of coat intact seed and naked embryos was tested under light (8/16 hours light and dark) and complete dark conditions. Dark germination of embryos was carried out only in selected experiments to reduce the workload on decoating. In each test, germination counts were taken for the first two weeks and the data were analysed statistically subjected to analysis of variance (ANOVA) using Minitab (Version 14).

4.3.1.3 Results

Effect of seed coat

Results showed that the seed coat reduced germination of guayule seed under light (Table 4.1) and in complete darkness (Table 4.2). Removal of the seed coat increased germination from 4.6% to 11.8% under light. Out of six different seed lots tested three had significant differences in germination between seed and embryos. The other three lots tended to produce slightly higher germination for embryos than seed but differences were not significant. Figure 4.1 shows the germination relationship between seed (with coat intact) and embryos in the presence of light for seed lots harvested during summer (February), autumn (April) and spring (October). These two factors show a high correlation with an R^2 of 0.94 with the relationship described by the equation;

$$\mathbf{Y = 0.97X + 10.63}$$

Where,
Y = Germination Percent of Embryo
X = Germination Percent of Seed

According to the equation, about 11% of germinable seed were unable to germinate due to the presence of the seed coat.

Seed coat had a great effect on decreasing germination in the absence of light (Table 4.2). Results showed that 47% to 68% of seed unable to germinate due to the presence of seed coat in complete darkness.

Table 4.1 Germination of guayule seed and embryos with variable harvest dates and age under 8/16 hours of light and dark

Seed harvest date	**Age of seed**	**Viability**	**Germination (%)**			**Difference**	**P value+**
			Seed		**Embryo**		
			Actual	**Adjusted**			
20/02/2004	19 days	75	64.0	84.7	95.0	10.3	0.022
20/02/2004	27 days	75	62.0	82.4	87.3	4.6	0.693
20/02/2004	40 days	78	57.3	73.5	79.3	5.8	0.236
07/04/2004	35 days	78	44.7	57.3	68.0	10.7	0.038
07/04/2004	8 months	78	66.7	85.5	97.3	11.8	0.023
31/10/2004	32 days	86	74.0	86.0	94.0	8.0	0.166

+ P value <0.05 is significant between seed and embryo germination

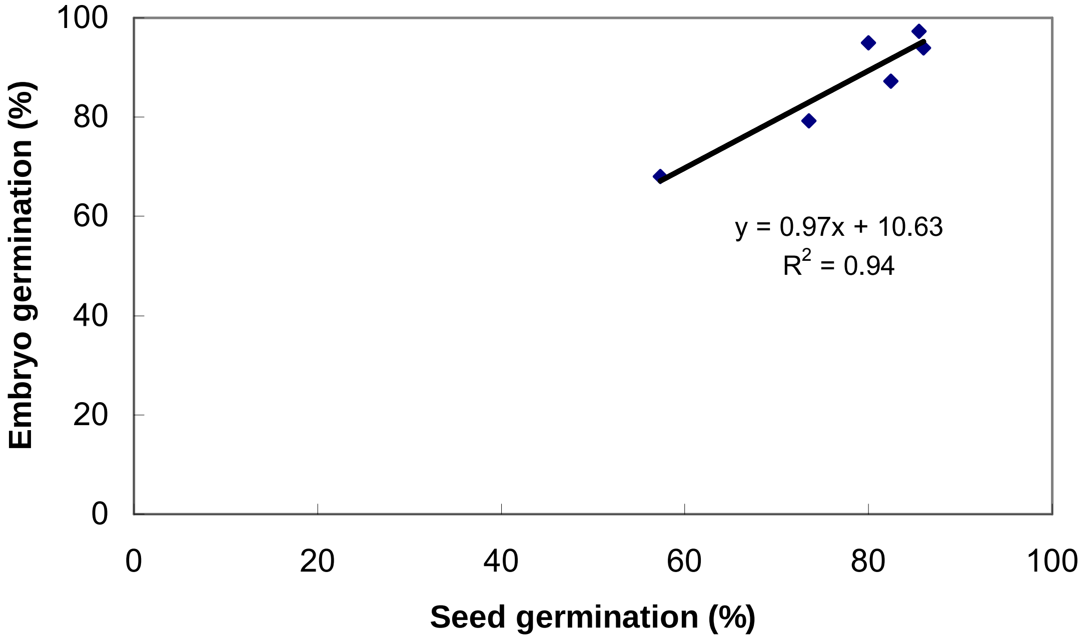


Figure 4.1 Relationship between embryo and seed germination for seeds of variable age groups

Extracted embryos not only had increased germination under light and complete darkness but germinated much quicker than coat intact seed (Figure 4.2). Embryos started germination on the second day and finished within 5 days whereas seed germination started about 3 to 4 days after soaking and continued to germinate till 9 to 12 days after soaking.

Table 4.2 Germination of seed and embryos with variable harvest dates and age under complete darkness

Harvest date	**Age of seed**	**Germination (%)**		**Difference as a percentage of embryo germination**	**P value+**
		Seed	**Embryo**		
20/02/2004	40 days	27.8	52.7	47.3	0.007
07/04/2004	35 days	11.0	34.7	68.4	0.014

+ P value <0.05 is significant between seed and embryo germination

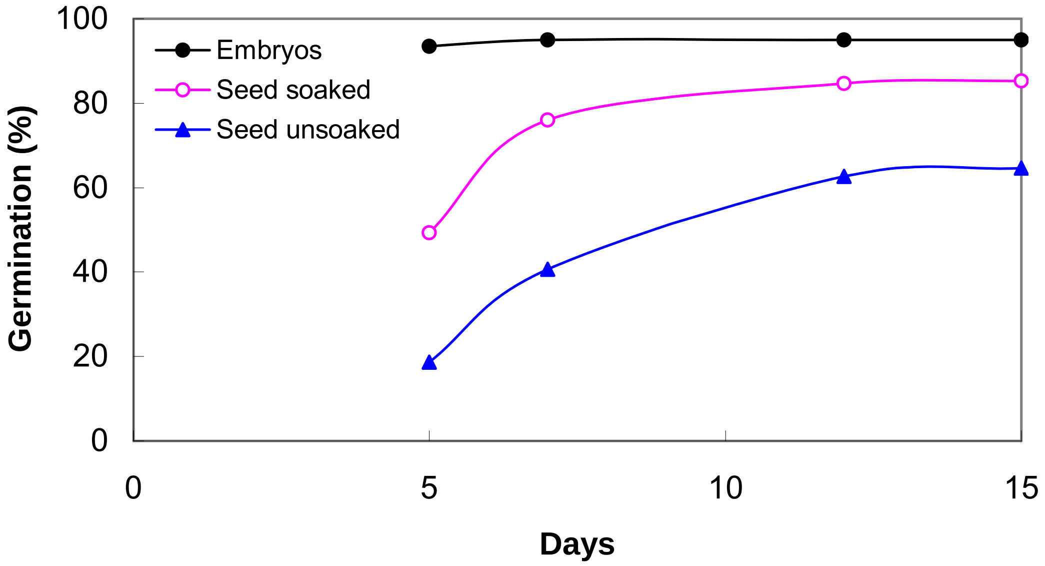


Figure 4.2 Trend in germination of 19 day old guayule seed and embryos harvested during late summer 2004

Viability of seed was relatively constant for time of harvest but there was a trend to higher levels with harvest in spring (Table 4.1). Dormancy levels appeared higher for seeds freshly harvested in autumn compared to freshly harvested seed in spring or summer.

Light effect

Exposure to light had a significant effect in increasing germination of fresh seed up to eight months of age (Table 4.3). The increase in germination due to light ranged from 67% to 85% and was significant for all six occasions. Light response appeared greatest for seed freshly harvested in autumn, i.e, when plants experienced low ambient temperature and shorter day length during seed maturation.

Table 4.3 Effect of light on germination of guayule seed with variable harvest dates and age

Harvesting date	**Age of seed**	**Seed germination (%)**		**Reduction in germination* (%)**	**P value+**
		Light	**Dark**		
20/02/2004	19 days	64.0	20.0	68.8	0.007
20/02/2004	40 days	57.3	16.7	70.9	0.001
20/02/2004	82 days	73.0	24.0	67.1	0.026
07/04/2004	35 days	44.7	6.7	85.0	0.001
07/04/2004	8 months	66.7	21.3	68.1	0.000
31/10/2004	32 days	74.0	21.3	71.2	0.002

+ P value <0.05 is significant between light and dark germination

* As a percentage of germination under light

Embryos had significantly higher germination under light (68% and 77%) than in the dark (34.7% and 52.7%) for two seed lots tested (Table 4.4). Differences between light and dark germination for embryos were narrower (31% to 49%) than coat intact seed (67% to 85%) (Table 4.5).

Table 4.4 Effect of light on germination of embryos extracted from guayule seed with two harvest dates but similar seed age

Harvesting date	**Age of seed**	**Embryo germination (%)**		**Reduction in germination* (%)**	P value+
		Light	**Dark**		
20/02/2004	40 days	76.7	52.7	31.3	0.009
07/04/2004	35 days	68.0	34.7	49.0	0.002

+ P value <0.05 is significant between light and dark germination

* As a percentage of germination under light

Table 4.5 Germination increased by removing the seed coat

Harvesting date	**Age of seed**	**Difference in germination with and without light (%)**		**Difference (coat effect)**
		Seed	**Embryos**	
20/02/2004	40 days	70.9	31.3	39.6
07/04/2004	35 days	85.2	49.0	36.2

Both light and seed coat had a significant effect on increasing seed germination of fresh guayule seed. The influence of light was much greater and produced difference of 67% to 85% for seed and 31% to 49% for embryos. Seed coat produced about 11% difference in germination in the presence of light. The difference was much greater (61% to 75%) in complete darkness.

4.3.1.4 Discussion

Influence of the seed coat
Both seed coat and light has a significant influence on germination of fresh guayule seed. In the presence of light, seed coat showed significant decrease in germination for some seed lots and for others the difference was non-significant. Variable results between seed lots could be due to environmental condition during seed maturation and storage. Cressnell and Grime (1981) and Benvenuti and Macchia (1997) reported with other species that parent environment during seed maturation play a key role in final dormancy.

Germination difference between seed and extracted embryos under light ranged from 4.6% to 11.8%. On average, about 11% seed failed to germinate due to presence of seed coat. The effect of seed coat was much higher in the absence of light (difference was between 61% to 75%). Removing of seed coat not only increase but produced faster germination than seed.

Benedict and Robinson (1946) argued that effect of seed coat is mainly due to impermeability to gas exchange. They also suggested that the presence of slight inhibitory action in the outer seed coat (fruit coat). Naqvi and Hanson (1982) found phenolic compounds in guayule chaff that inhibit seed germination. Results of the current study also strongly support the hypothesis of having inhibitory action in the seed coat. Because removing seed coat increased germination under complete darkness (from 21.4% and 8.6% to 48.3% and 34.7% respectively). Also seed always produced reduced germination with coat intact seed compared to embryos (on average about 11%). Seed coat also seems to have mechanical resistance to germination. Delayed seed germination over embryos indicates that seed coat act as a mechanical barrier. As described by Benedict and Robinson (1946) delayed germination could also be due to impermeability to gas exchange or could act as a mechanical barrier to emerging seedling. However, results of current study did not show vast difference as found by Benedict and Robinson (1946) where they reported to have 60% increased in germination by removing or damaging the seed coat. Variable results between the current study and the results of Benedict and Robinson (1946) could be due to the differences in genotype. Because Benedict and Robinson used seeds from some of the older lines developed during World War II. Seed used in the current study was obtained from a newly developed line of AZ-2.

Leubner-Metzger (2001) found that seed germination of *Nicotiana tabacum* L. cv. Havana 425 is determined by the balance of forces between the growth potential of the embryo and the mechanical restraint of the micropylar endosperm. He further discovered that gibberellin induce 1-3 glucanase in the micropylar endosperm to release coat-enhanced dormancy by rupturing the endosperm. Delayed germination from the current study for seed compared to embryos also could be due to requirement of endosperm rupture.

Influence of light

Results in Table 4.3 show that light is the most important determinant for overcoming dormancy of fresh guayule seed. Some 63% to 85% seed failed to germinate in the absence of light. Previous researches reported comparable results for light effect on germination. Using a number of seed lots varying from 2 to 10 months old, Benedict and Robinson (1946) found that 20% to 66% of seed unable to germinate in the absence of light. Hammond (1959) reported that freshly harvested seed produced 9.5% seed germination in the dark and 79.1% under light after 22 days, that is, 88% of seed failed to germinate with absence of light.

Embryos extracted also showed a great reduction in germination (31% and 49%) in absence of light (Table 4.4). However, removing seed coat reduced the gap between light and dark germination indicating the presence of inhibitors in the seed coat (Table 4.5).

Results further indicate the influence of environmental conditions during seed maturation on dormancy. During summer (February) and mid spring (October), plants were under favorable ambient temperature, day length and soil moisture. These favorable conditions optimized the plant growth and hence seed maturity. Therefore, seed produced during these periods were comparatively less dormant. In autumn (April), plants experienced low temperature, shorter day-lengths and high soil moisture stress. Seed produced during this period had high levels of dormancy with germination in the dark (6.7%) as well as under light (44.7%). The difference between light and dark germination was also high (85%) compared to seed produced during favourable environmental condition (67% to 71%) and indicates high levels of inhibitors and/or low levels of growth promoters. This indicates that plants ecological adaptation to environmental conditions where plants produce seed with high levels of dormancy to ensure that they would not germinate to face undesirable weather (cold temperature) in autumn and winter. This hypothesis supported by Benvenuti and Macchia (1997) finding that the balance between stimulators, such as Pfr, and inhibitors (abscissic acid and or phenolic substances) in the stage prior to complete dehydration play a crucial role in the degree of progeny dormancy.

Some older seeds reported to respond to light in germination. Chandra (1991) found that even six years old osmo-primed seed (by polyethylene glycol) responded to light (31% in the dark and 93% under light). This could be due to secondary dormancy imposed by the environmental condition during storage.

Results strongly support the hypothesis of regulating guayule seed germination by the balance of growth promoter and inhibitor balance. Seed coat too has inhibitors that affect this balance. Further maternal environmental conditions during seed maturity appeared to decide the level of progeny seed dormancy. Further these factors of seed coat dormancy, embryo dormancy and light effect appeared to have interactions between them.

4.3.2. Effect of red light, gibberellic acid, potassium nitrate and prechilling in overcoming seed dormancy

4.3.2.1 Introduction

Various seed treatments have been used to overcome dormancy in plant species. Light and its quality are important factors for germination of some species that responded to light (Bewley and Black, 1994). Of the visible spectrum of light, red light is the most important radiation to induce germination for many small seeded species. Gibberellic acid is one of the hormones that can help in hydrolyzing reserves in seed to activate germination (Bewley and Black, 1994; Taiz and Zeiger, 2002). Nitrate compounds also interact with light to induce seed germination in many species (Batak *et al*, 2002). Chilling or exposure to low temperature is another factor inducing seed germination in some species (Watkinson and Pill, 1998; Bourgoin and Simpson, 2004).

Therefore, a pilot study was undertaken to check whether these individual factors and their combinations had an effect in overcoming seed dormancy with a view to later conducting a replicated trial using effective treatments.

4.3.2.2 Materials and method

A seed germination test was carried out to investigate the effect of various treatments on overcoming seed dormancy in guayule. In late summer (2004), 19 days old seed was harvested for this test. Treatments used in this experiment were:

- exposure to red light
- gibberellic acid
- potassium nitrate
- prechilling and
- combinations of above

For germination test, a sample size of 50 seed was selected without replication.

Seeds were soaked overnight between paper towels before chilling and then transferred to petri dishes with three layers of wet filter papers. These petri dishes were later chilled for 4 days at 5°C in a refrigerator just before the germination test. Seeds not meant for prechilling were soaked overnight a day before the test. Then these soaked only seeds and soaked and prechilled seeds were transferred to petri dishes with filter papers wetted by demineralised water (control and red light) and chemicals (gibberellic acid, KNO_3 and combinations of them). A red florescent bulb was used to provide 15 minutes exposure to red light just before the test. A germination test was conducted under 8/16 hours light and dark at 20°C for 13 days and the results obtained were used to plan further tests.

4.3.2.3 Results

Germination of seed exposed to 15 minutes of red light (50.0%) was similar to that without exposure (48.0%) (Table 4.6). However, both gibberellic acid treatment (68.0%) and prechilling (64.0%) alone appeared to increase seed germination compared to the control (48.0%). KNO_3 also appeared to increase germination (60.0%) but the response was slower than for gibberellic acid and prechilling alone (Figure 4.2). Prechilled and GA_3 treated seed finished germination by 9th day. However KNO_3 treated seed continued to germinate until the end of second week.

Table 4.6. Germination of seed and embryos under various seed treatments

Seed treatment	**Germination (%)**		
	Seed		**Embryos**
	Actual	**Adjusted for viability**	
No treatment	48	64.0	90.0
15 min red light	50	66.7	84.0
GA$_3$	68	90.7	92.0
KNO$_3$	60	80.0	78.0
GA$_3$ + KNO$_3$	64	85.3	94.0
Prechilled (4 days/5°C)	64	85.3	90.0
Prechilled + GA$_3$	72	96.0	88.0
Prechilled + KNO$_3$	58	77.3	92.0
Prechilled + GA$_3$ + KNO$_3$	62	82.7	92.0

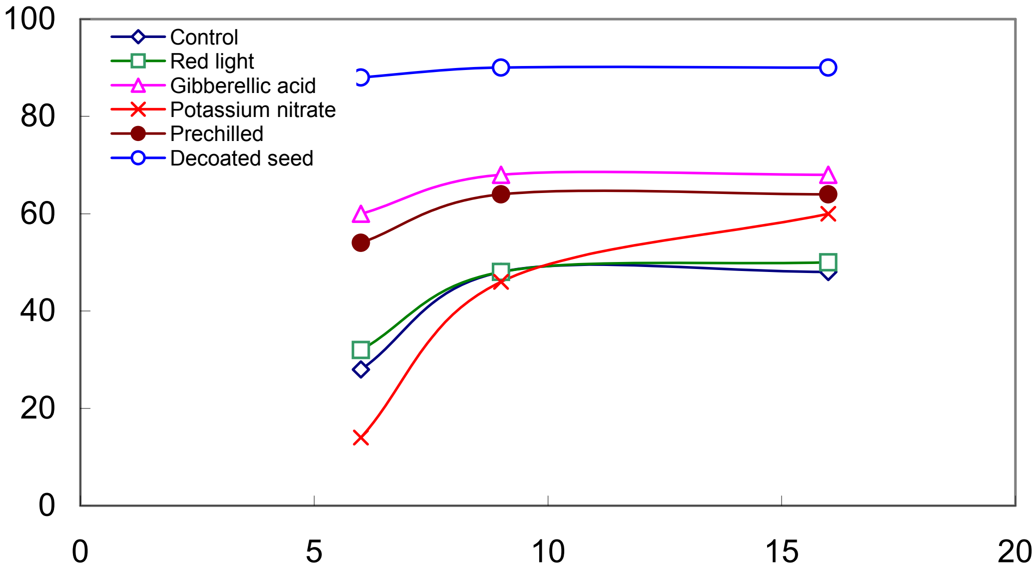


Figure 4.2 Trends in seed germination of treated and untreated guayule seed and embryos under light

Germination from treatment combinations appeared similar to individual treatments of gibberellic acid or prechilling. Extracted embryos always had higher germination (84.0% to 92.0%) than coat intact seed irrespective of seed treatment and were quite high for embryos without treatment.

4.3.2.4 Discussion

Under eight-hour light, individual treatments of gibberellic acid, chilling and KNO$_3$ appeared to be effective in breaking dormancy in fresh guayule seed. Of these treatments, gibberellic acid and chilling appeared most effective because germination was faster compared to KNO$_3$ treatment. Short duration exposure (15 min) of seed to red light had no effect on increasing seed germination. Under light, embryos produced high germination and therefore, treatments used had no apparent effect on germination

4.3.3 Effect of light quality on guayule seed germination

4.3.3.1 Introduction

Seeds of some species that are sensitive to light require a specific light quality to induce germination. Red light found to be the most important radiation in the visible spectrum of light for many seed in breaking dormancy. The effect of red light is mediated through phytochrome (Bewley and Black, 1994; Smith, 2000). It stimulates or inhibits germination according to the level of red or far-red radiation in the light. Generally, light with high levels of far-red light (light filtered through a plant canopy) inhibits germination, while light with high levels of red light stimulate germination (Gorski, 1975; Taiz and Zeiger, 2002).

While some species affected by brief exposure to light others require intermittent illumination or require exposure to long days to induce germination (Bewley and Black, 1994; Taiz and Zeiger, 2002).

Light has been shown in this and other studies to be very important for germination of fresh guayule seed. However, the effect of light on germination of guayule seed still remained undiscovered. Therefore, the objective of this experiment was to investigate whether the quality of radiation has any effect on germination in guayule seed.

4.3.3.2 Material and method

Two experiments were conducted to investigate the effect of light quality on germination. 10 months old seed harvested during November 2003 and seed harvested during October 2004 (32 day old) were tested for germination after daily exposure of 8 hours to light of different qualities.

In experiment one, germination test for 10 moths old seed harvested during November 2003 was carried out using the following light treatments:

- Normal
- Green
- Red and
- No light (dark)

The coloured light was obtained by wrapping petri dishes in two layers of colorized cellophane paper.

Initially, seed was soaked between paper towels and then transferred to 10 cm glass petri dishes with three layers of filter papers wet by demineralised water. Immediately these petri dishes were wrapped with coloured cellophane paper and placed in germination cabinets with normal white light (under florescent light). The germination test was carried out for 14 days at which the number of seed germinated was counted.

The second experiment was conducted with 32 days old seed harvested during October 2004 from the same plants. This experiment was conducted in a similar way, but using dditional light treatments of blue and yellow. Germination counts were taken 13 days after starting the test. Data obtained from both tests were analysed statistically using Minitab (Version 14).

4.3.3.3 Results

Light quality had a significant effect on germination of freshly harvested seed of 32 days of age (Table 4.8). Four out of five types of light, green (55.3%), red (65.3%), yellow (82.0%) and normal white light (76.0%) significantly increased germination compared to no light (dark) (21.3%) while blue light had no significant effect (25.3%). Yellow light produced the highest germination and was significantly higher than that for green light. However, germination was not significantly different for yellow, red or normal white light. Results showed that yellow and red were the two important colours of light in the visible light spectrum that induce seed germination in fresh guayule seed. Figure 4.3 shows the trend in germination against wavelength of light and indicates that radiation within wavelength from 550 nm to 680 nm is the most effective in inducing germination.

Results obtained with 10 months old seed showed that exposure to normal white (69.4%) or to different coloured light (red 54.6% and green 62.0%) had no significant effect on increasing germination (Table 4.7).

Table 4.7 Effect of different colour light on germination of 10 months old guayule seed harvested during summer 2003 (November)

Treatment	Mean germination (%)
Dark	56.6 a
White light	69.4 a
Red light	54.6 a
Green light	62.0 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Table 4.8 Effect of different colour light on germination of 32 days old guayule seed harvested during spring 2004 (October)

Treatment	Wave length (nm) for different colour band in the visible spectrum+	Germination
Dark	0	21.3 c*
Blue light	435 - 500	25.3 c
Green light	520 - 565	55.3 b
Red light	625 - 740	65.3 ab
Yellow light	565 - 590	82.0 a
White light	380 - 740	76.0 ab

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

\+ Wavelength for relevant colour band

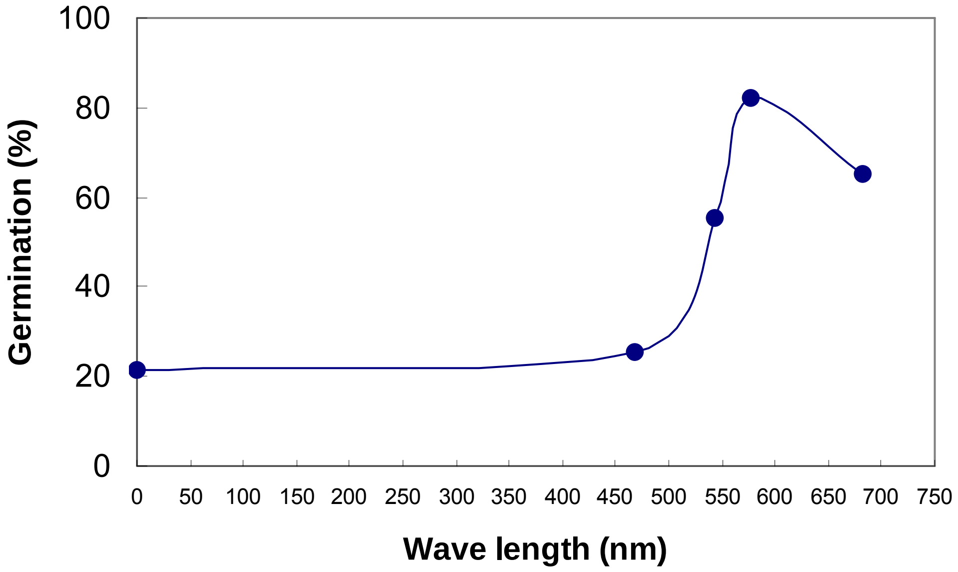


Figure 4.3 Effect of different colour light on germination of 32 day old guayule seed harvested during spring 2004 (October)

4.3.3.4 Discussion

Results (Table 4.3 and Table 4.4 in section 4.3.1) show that light is a major factor deciding germination of fresh guayule seed. Table 4.7 shows that not only exposure to light, but its quality is also important for overcoming seed dormancy. Results of Table 4.6 show that light has no significant effect in increasing germination of 10 months old seed. Therefore, it is clear that this seed lot has completed its dormant period. However, some seeds as old as 6 years responded to light by germinating (Chandra, 1991). These results indicate that light is always important for fresh guayule seed to overcome dormancy. Differential response by older seed indicates that either parental environmental conditions and/or seed storage conditions has an effect on changing the balance of growth promoter and inhibitor in the seed.

Yellow and red radiation within 550 nm to 680 nm wavelength range is most effective in inducing germination of guayule. The effect of yellow light on germination was observed with other species. Ahlawat *et al*. (1979) reported that seed germination of *Parthenium hysterophorus* inhibited under continuous red, blue, green and white light but not under yellow light.

The normal white light produced similar result as yellow and red light. This could be due to the presence of required levels of effective radiation or ratio of red to far-red radiation in white light.

These results provide strong evidence about the presence of phytochrome in the embryo that mediates germination in guayule. Increased germination with red and yellow light compared to green light produces strong evidence of phytochrome-mediated germination. As this is normally governed by the ratio of red to far-red radiation, a higher ratio of red to far-red induces seed germination while increased levels of far-red radiation inhibit germination. Phytochrome-mediated germination generally produces low gemination under green light (radiation filtered through a plant canopy) due to comparatively high levels of far-red to red light as described by Gorski (1975). The current study also obtained comparable results by producing significantly lower germination under green light than that of yellow and slightly lower than red and white light.

Further, the inhibitory effect of blue light on germination could be due to the low levels of red radiation to inactivating phytochrome. Burritt and Hunter (2004) found that lettuce cotyledon explant exposure to blue light within 7 days of excision found to inhibit shoot production. Therefore, low germination under blue light could also be due to inhibiting the shoot growth.

Results of Table 4.3 and 4.4 in section 4.3.1 show that light is a major factor deciding whether fresh guayule seed would germinate or not. The data also show that there was a difference in response to light by different seed lot. This supports the finding by Cressnell and Grime (1981) of the influence of maternal environment on seed dormancy during seed maturation.

4.3.4 Effect of gibberellic acid and abscisic acid on germination

4.3.4.1 Introduction

Seed germination is believed to regulated by the two primary hormones, gibberellin and abscisic acid (Ni and Bradford, 1993). Embryo dormancy is thought to be due to the presence of inhibitors, especially abscisic acid (ABA), as well as the absence or low levels of growth promoters, such as gibberellic acid. ABA inhibits the synthesis of hydrolytic enzymes essential for the breakdown of storage reserves in seeds. The loss of embryo dormancy is often associated with a sharp drop in the ratio of ABA to gibberellic acid (Taiz and Zeiger, 2002). The hormone balance theory proposed that the relative amounts of gibberellins and abscisic acid determined whether a seed would germinate.

External addition of growth promoters and inhibitors changes the growth promoter and inhibitor balance to induce or inhibit germination (Grappin *et al.*, 2000). Past research on the effect of gibberellic acid on germination of guayule seed has produced variable results. Therefore, the objectives of this were to investigate whether guayule seed dormancy is governed by the balance of growth promoters and inhibitors and determine whether gibberellic acid could totally replace the light requirement in germination.

4.3.4.2 Materials and method

Three experiments were carried out to investigate the effect of gibberellin, its concentration and abscisic acid on guayule seed germination.

Test 1- Effect of GA_3

35 days old seed harvested on the 7 April 2004 (autumn) was used. Both seed and embryos treated with gibberellic acid were tested for their germination under light and in complete darkness and compared with an untreated control. Embryos and coat intact seed were placed in separate 10 cm petri dishes having three layers of filter papers wetted with 500 ppm gibberellic acid. Germination of these seeds was tested under 8 hour light and in complete darkness at 20°C. In the control, blotters were moistened with demineralised water. Germination counts were taken up to 13th day.

Test 2- Effect of concentration of GA_3

The test used 32 days old seed harvested on the 31 October 2004 (spring). Seed was soaked overnight between paper towels before the test. Then they were placed in separate petri dishes having filter papers wetted with different concentrations of gibberellic acid (50 ppm, 150 ppm and 250 ppm). In the control, blotters were moistened with demineralised water. Germination tests were carried out in 8/16 hour light and dark as well as complete dark conditions. Germination counts were taken after 13 days.

Test 3- Effect of GA3 and ABA
A third experiment was conducted in a similar way to the above experiments using abscisic acid and 250 ppm giberrelic acid but without extracted embryos and without the dark treatment. Results obtained from all three experiments were analysed statistically.

4.3.4.3 Results

Germination Test 1
Germination of seed and embryos in the dark (8.6% and 34.7% respectively) and under light (57.3% and 68.0% respectively) produced variable results for the untreated control (Table 4.9). Treatment with 500 ppm gibberellic acid significantly increased seed germination in all treatments. It was very effective in both dark and light germination and also on seed with or without a seed coat. Almost 100% germination was produced in all cases.

Further Table 4.10 shows these three factors of light, GA and seed coat are highly interactive except seed coat and light. These results support the argument of seed dormancy being controlled by the balance of growth promoters and inhibitors.

Table 4.9 Effect of gibberellic acid on germination of intact seed and embryos with and without light

Source	Light	**Germination mean (%)**	
		Control m+	**Gibberellic acid (500 ppm)**
Seed	Dark	8.6 a	94.0 a*
	Light	57.3 c	94.9 a
Embryo	Dark	34.7 b	91.3 a
	Light	68.0 d	96.0 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.
+ Seed germination was adjusted for their viability of 78%

Table 4.10 Analysis of variance for germination under various treatments of light versus dark, seed versus embryo and with and without gibberellic acid.

Source	**P value**
GA_3	0.000
Light (Light Vs Dark)	0.000
Light x GA_3	0.000
Light x Seed coat	0.156
Light x Seed coat x GA_3	0.013
Seedcoat (Seed Vs Embryo)	0.000
Seedcoat x GA_3	0.000

Germination Test 2
Gibberellic acid concentration had a significant effect on dark germination of fresh guayule seed (Table 4.11 and Figure 4.5). All three concentrations of gibberellic acid, 50 ppm (63.4%), 150 ppm (76.0%) and 250 ppm (86.0%), significantly increased germination of 32 day old seed in complete darkness compared to the control 21.4%. Results also showed that 250 ppm gibberellic acid produced significantly higher germination than 50 ppm GA. Germination of seed treated with 250 ppm slightly higher than 150 ppm however the difference was not significant.

Under light, gibberellic acid had no significant effect on germination; however, higher concentrations (150 ppm and 250 ppm) tended to produce slightly higher values (83.4% and 85.4% respectively) compared to the control (76.4%). Further Table 4.12 shows high interaction between light and GA_3 in seed germination.

Table 4.11 Effect of light and gibberellic acid concentration on germination of 32 days old guayule seed

Light/Dark	**Treatment**	**Mean Germination (%)**
Dark	250 ppm GA_3	86.0 a
	150 ppm GA_3	76.0 ab
	50 ppm GA_3	63.4 b
	Control	21.4 c
Light	250 ppm GA_3	85.4 a
	150 ppm GA_3	83.4 ab
	50 ppm GA_3	78.0 ab
	Control	76.0 ab

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Table 4.12 Analysis of variance for germination under treatments of light and gibberellic acid.

Source	**P value**
Light	0.000
GA_3	0.000
Light x GA_3	0.000

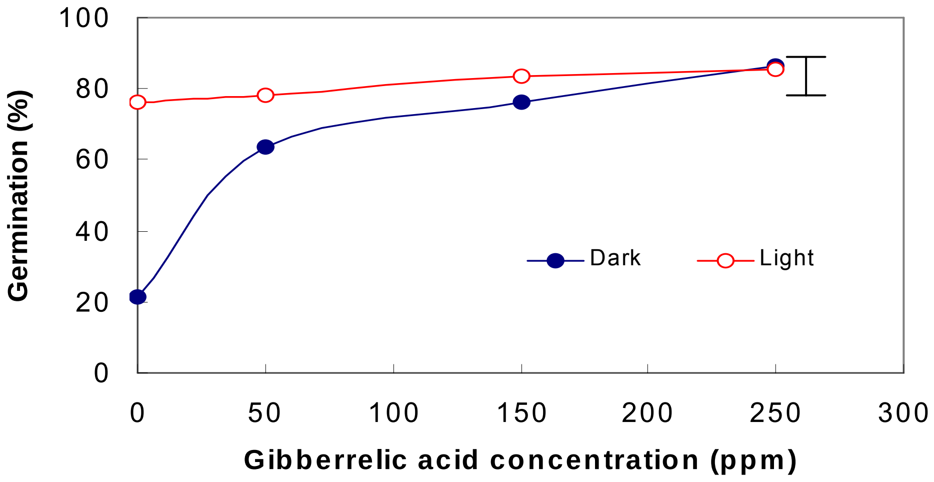


Figure 4.5 Germination behaviour of guayule seed with increasing concentrations of gibberellic acid in dark and under light

Germination Test 3

Addition of 100 µM abscisic acid almost stopped germination of seed (1.4%) while gibberellic acid (74.6%) produced similar results to the control (77.4%) (Table 4.13). Therefore, addition of external inhibitors has the ability to restrict germination. Similar results obtained by Ni and Bradford (1993) using tomato seed. In addition to 100 µM, they also used lower concentration that they obtained lower partial inhibition. Further, results from current study, it was also noticed that seedling emerged in ABA treated seed did not survived.

Table 4.13 Effect of gibberellic acid and abscisic acid on germination of 32 days old guayule seed

Treatment	**Germination (%) (viability 83%)**
Control	77.4 a
250 ppm GA_3	74.6 a
100 μM ABA	1.4 b

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

4.3.4.4 Discussion

Gibberellic acid has a great influence on the germination of guayule seed. It can produce almost 100% seed germination in light as well as under dark conditions by treating seed with the correct concentration. Ni and Bradford (1993) obtained similar results with tomato seed. They further found that GA_3 concentration is important to overcome seed dormancy.

Table 4.14 shows a summary of data obtained from the literature, showing the use of different concentrations of gibberellic acid to break seed dormancy in guayule. Hammond (1959) concluded that GA_3 can totally be replaced the light requirement by using a high concentration of 1000 ppm. Naqvi and Hanson (1980) found 200 ppm GA_3 was not enough to produce 100% germination. However, the combined effect of 200 ppm GA_3 and NaOCl was able to produce 100% germination in dark condition. These results indicate that GA_3 and NaOCl have an additive effect when the concentration of GA_3 on its own was not sufficient to completely overcome dormancy. It further revealed that NaOCl had reduced the inhibitory effect to some degree perhaps by oxidizing inhibitors in the seed coat and/or in embryo. This study confirms those previous reports that GA_3 can totally replace the light requirement provided the concentration is high enough.

Table 4.1 in section 4.3.1 shows that the level of dormancy varies with specific seed lot. Therefore required concentration of GA_3 depends on the specific seed lot in particular how old it is and parental environmental condition during seed maturation. Studying seed dormancy in *Arabidopsis* seed Debeaujon and Koornneef (200) suggested that GAs are required to overcome germination constraints imposed by the seed coat and ABA-related embryo dormancy. Leubner-Metzger (2001) found with tobacco seed that gibberellin induce β-I-3 glucanase in the micropylar endosperm to rupture endosperm and release of coat enhanced dormancy. He further suggested that gibberellins and light act in a common pathway to release photodormancy in tobacco seed.

Ni and Bradford (2000) found with tomato seed that a change in the threshold levels of ABA and GA_3 was the primary factor determining germination or dormancy. They also concluded that endogenous or exogenous growth regulators can shift the threshold level and that they determine both the rate and final extent of germination within the seed population.

The inhibition of germination by external ABA was reported with many other species. Ni and Bradford reported that treating tomato seed with μ100 M ABA almost stop germination. Paiva (1995) discovered that abscisic acid inhibits the precocious germination during process of embryo development. Garciarrubio *et al*., (1997) found that addition of ABA to mature non-dormant

Table 4.14 Effect of gibberellic acid concentration and light on guayule seed germination from previous research

Author and year	Light/ Dark	Control	Seed treatment	Germination of treated seed
Hammond, 1959	Light	83.5	1000 ppm GA_3	88.5
Naqvi and Hanson, 1980 (fresh seed)	Light	52.0	200 ppm GA_3	72.0
	Light	52.0	200 ppm GA_3 + 1% NaOCl	97.0
Naqvi and Hanson, 1980 (one year old)	Light	63.0	200 ppm GA_3 + 1% NaOCl	98.0
Chandra, 1991 (6 year old conditioned seed)	Dark	30.8	35 ppm	80.8
	Light	93.0	35 ppm	94.6

arabidopsis seed inhibit germination by preventing the degradation of the seed storage proteins. They suggest that ABA inhibit seed germination by restricting availability of energy and metabolites.

Results of this study also showed that exogenous ABA totally inhibited the germination of guayule seed. Therefore, results obtained by treating seed with GA_3 and ABA (Table 4.13) together with results from previous experiments strongly support the claim of controlling seed germination by the balance of growth promoters and inhibitors.

4.3.5 Effect of washing, NaOCl and soaking time in overcoming seed dormancy

4.3.5.1 Introduction

Washing of seed in water or treating seed with NaOCl is effective in overcoming seed coat imposed dormancy in some species. Washing is effective in leaching out inhibitory chemicals in the seed coat and it also softens the seed coat to allow embryo to develop (release seed from dormancy). Treating seeds with NaOCl is also effective in breaking seed dormancy through oxidizing the seed coat to make it soft or permeable (Emparan and Tysdal, 1957; Hammond 1959; Naqvi and Hanson, 1980; Hsaio and Quick, 1984). Therefore, germination tests were carried out to investigate the effects of washing and NaOCl in ovecoming seed dormancy in guayule.

4.3.5.2 Materials and method

Three experiments were conducted to investigate the effect of washing, soaking time and NaOCl in breaking seed dormancy. In test one, 19 day old seed harvested in late summer was used to investigate the effect of seed washing and treatment with 1% NaOCl. These treatments were compared with an untreated control. For washing, seeds were wrapped in a cotton cloth and placed in a beaker. Then tap water was allowed to continuously flow through the beaker for 8 hours. In the other treatment, seed was soaked in 1 % NaOCl for 2 hours and then rinsed seed with deionised water to remove residual chemical.

Seed used in the second test was harvested at the same time as that used in test one but the age was 27 days at the start of the test. In this test the effect of soaking times, 4 and 12 hours, were compared with treatment with 1% NaOCl. Seed used in the third test was also harvested at the same time as above two tests and the age of seed was 40 days at the beginning of the test. In this test, soaking time of 4 and 12 hours were compared with an unsoaked control. Germination counts in all three tests were recorded during first two weeks and data were analysed statistically using Minitab (version 14).

4.3.5.3 Results

Germination test 1

Germination of seed after eight hours washing in running tap water (48.0%) was significantly lower than seed soaked overnight between paper towels (control - 63.3%) (Table 4.15 and Figure 4.6). Therefore, seed washing in running tap water was less effective than soaking them between paper towels. Extracted embryos showed no significant differences among treatments and always produced higher germination (93% - 95%).

Table 4.15 Effect of washing and NaOCl on germination of seed and embryos

Treatment	**Germination (%)**	
	Seed (Viability 75%)	**Embryos**
Control	63.3 a	95.0 a
8 hr washing in running tap water	48.0 b	93.3 a
2 hr treatment in 1% NaOCl	62.7 a	95.3 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

NB. Seeds used for 8 hours washing and NaOCl treatment did not soak overnight and all other seeds soaked overnight between paper towels.

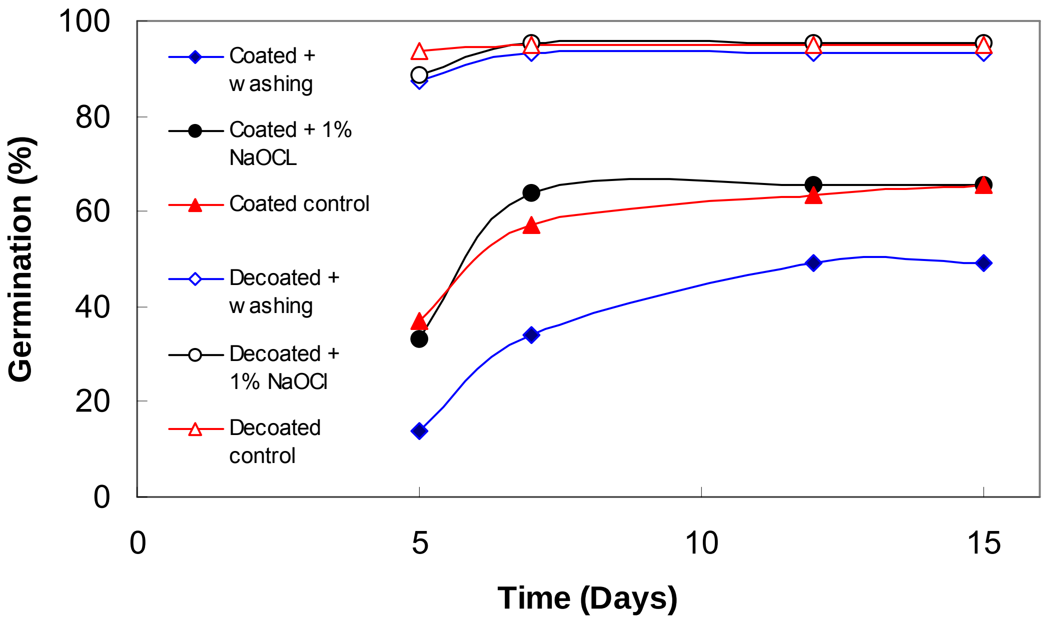


Figure 4.6 Effect of washing and NaOCl on germination behaviour of guayule seed and embryos

Germination test 2

Treatment with 1% NaOCl for 2.5 hours and soaking seed for 4 to 12 hours had similar effects on seed germination (Table 4.16). Embryos always had higher germination and different treatment showed no significant difference on germination of embryos. Germination of embryos treated in the same way showed no significant differences.

Table 4.16 Effect of seed soaking time and NaOCl on guayule seed germination

Soaking	**Treatment**	**Gemination (%)**	
		Seed (viability 75%)	**Embryo**
4 hour	No	63.3 a	86.7 a
12 hour	No	62.0 a	87.3 a
No soaking	2.5 hr in 1% NaOCl	62.7 a	87.3 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Germination Test 3

Germination of seed soaked for 4 and 12 hours was not different to that for unsoaked seed (Table 4.17). However, it was observed there was some delay in achieving final germination with unsoaked seed (Figure 4.7).

Table 4.17 Effect of seed soaking time and NaOCl on germination seed and embryos in dark and under light

Soaking	Germination (%)			
	Coat intact seed (Seed viability 78%)		Extracted embryos	
	Dark	**Light**	**Dark**	Light
Unsoaked	20.7	60.0	NA	NA
4 hr soaking	26.7	59.3	50.0	78.0
12 hr soaking	16.7	57.3	55.3	84.6

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

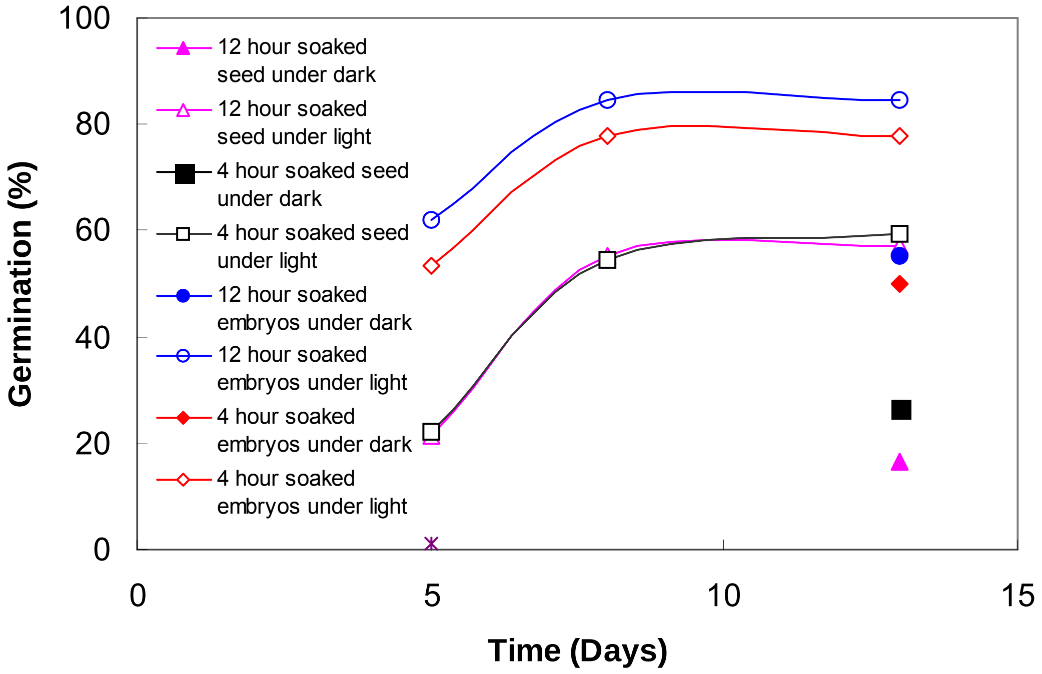


Figure 4.7 Germination behaviour of guayule seeds and embryos with variable soaking time and light conditions

4.3.5.4 Discussion

Seed soaking was more effective than washing in increasing seed germination. This could be due to presence of water insoluble inhibitors in the seed coat. These inhibitors may have being absorbed by paper towels while soaking. Washing may not be leaching out inhibitors since they are water insoluble.

Treating with 1% NaOCl was as effective as soaking to increase seed germination. As described by Naqvi and Hanson (1980) the oxidising action of NaOCl may help in reducing inhibitors in the seed coat to increase germination.

A short soaking time of 4 hours was as effective as 12 hour soaking. This indicates that four hour soaking between paper towels was enough to leach out sufficient amounts of inhibitor to increase germination.

4.3.6 Effect of prechilling on germination

4.3.6.1 Introduction

Low temperature or chilling is a method used to release seed from dormancy in some species (Watkinson and Pill, 1998; Taiz and Zeiger, 2002; Bourgoin and Simpson, 2004; Heide and Prestrud, 2005). Many seeds require a period of cold (0 to 10°C) while in the fully hydrated state in order to germinate. In temperate-zone species, this requirement is of obvious survival value, since such seeds will not germinate in the autumn but in the following spring (Taiz and Zeiger, 2002).

The results of pilot study also showed that chilling was effective in increasing seed germination. Therefore, the objective of this study was to investigate the effect of chilling on breaking seed dormancy.

4.3.6.2 Materials and method

54 day old seeds harvested in autumn 2004 were used. Seeds were initially soaked overnight between paper towels. Then they were transferred to petri dishes with filter papers wetted by demineralised water. Then petri dishes were placed in a plastic bag and refrigerated for 5 days at 5°C. Seed to be used as control soaked a day before the test and then they were transferred to petri dishes with wet filter papers. Germination tests of these prechilled and untreated controls were carried out under eight-hour light and in complete darkness. Germination data were recorded for 15 days and they were analysed statistically.

4.3.6.3 Results

Results showed that chilling had no significant effect on germination under light (68.7%) even though there tended to be a slight increase (74.7%) (Table 4.18 and Figure 4.8). Chilling produced a significant effect on increasing seed germination in complete darkness (12.0% vs 2.7%). However, the level of increased germination was not as effective as exposure to light or gibberellic acid treatment.

Table 4.18 Effect of prechilling on germination of guayule seed with and without light

Treatment	**Light/Dark**	**Germination (%)**
Soaked only	Dark	2.7 a*
Soaked and prechilled	Dark	12.0 b
Soaked only	Light	68.7 c
Soaked and prechilled	Light	74.7 c

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey’s simultaneous test.

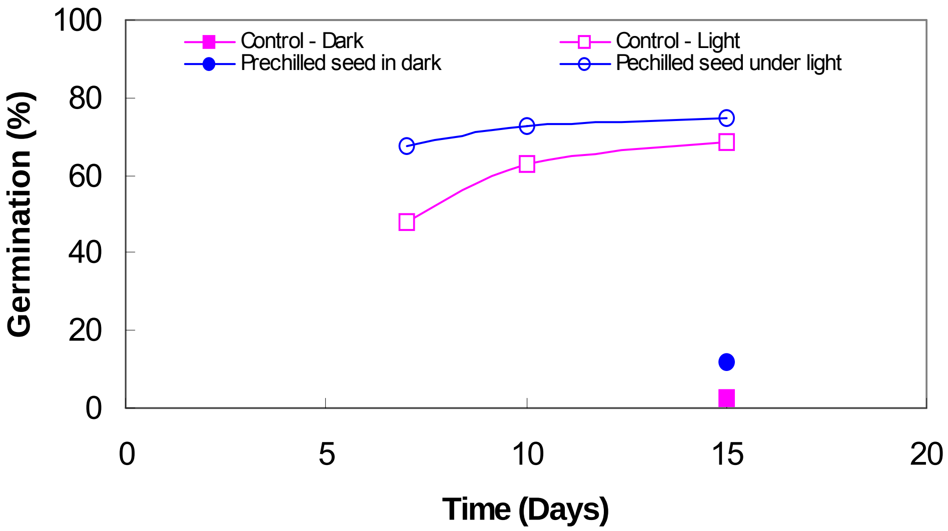


Figure 4. 8 Germination behaviour of prechilled guayule seed with and without light compared to untreated seed

4.3.6.4 Discussion

Benedict and Robinson (1946) reported that prechilling of guayule seed at 4-5°C for various periods from 5 days to 60 days had no significant effect on germination. However, their experiments were conducted only under light and they did not indicate whether chilling was employed in fully hydrated state or not.

In the current study prechilling was carried out for hydrated seed under light as well as under dark conditions. Results showed that prechilling significantly increased germination in dark conditions but there was no significant effect under light. However, results show that prechilling was not as effective as exposure to light or treating with GA_3 from earlier experiments. The increase of germination in dark conditions could be due to decrease in inhibitory action by prechilling but it was only partially effective.

5. Direct seeding of guayule

5.1 Introduction

High cost of establishment is a primary factor restricting the commercialisation of guayule. The current widely used and reliable method of establishment is transplanting by greenhouse grown seedlings. This is the most expensive plant production practice of guayule. Transplanting was estimated to cost A$ 1800 per hectare in Australia (Jackwitz, 2003) and US$ 1600 per hectare in the U.S. (Foster *et al*. 2004). Therefore, establishment is an important aspect that needs to be addressed in commercialisation of guayule. Development of a direct field seeding method would in reduce production costs substantially. The objective of this study was to investigate direct seeding of guayule as an alternative to transplanting.

5.2 Materials and method

Two field trials were conducted on the research farm of the Gatton Campus of University of Queensland. The first trial was carried out during October - December 2002 and the second during November-December 2004. The soil characteristics are as described in Chapter 3.

5.2.1 Experiment 1

Seed harvested from line AZ-3 from the Gatton germplasm evaluation trial were used. Seeds were air-dried, then threshed in a F.Walter – H.Wintersteiger KG, Austrian made thresher. Cleaning carried out by manually using 1.18 mm and 2.36 mm sieves and using a KamasWestrup type LALS gravity table. All foreign matter was manually removed and a laboratory seed blower used to remove unfilled and partially filled seed. Cleaned seed was then treated by the method outlined by Chandra (1991) to break seed dormancy and improve emergence and establishment. This osmo-conditioning method involves imbibing seeds in a medium containing polyethylene glycol (PEG), gibberellic acid, potassium nitrate and thiram (tetramethylthiuram disulphide). Treated seeds were divided into three equal lots. One third was left without further treatment as control, the second was lime coated by Heritage Seed Pty Ltd. The lime coat included almost all range of macro and minor nutrients. The last third was sent to Livyn Pvt. Ltd. to include them in tapes (Figure 5.1).

Figure 5.1. Types of seed, conditioned (control), conditioned and lime coated and conditioned and taped used for direct seeding

Land preparation for field planting was carried out in three steps. Tine cultivation followed by two harrowings by a tractor-mounted rotavator. Finally, the field was rolled to obtain a uniform seedbed. The experimental design was a randomised complete block with four replicates. Plot size was a single row 8 m in length rows were one meter apart. A hand shift sprinkler system was used to irrigate the field.

A seed germination test was carried out just before field seeding. The field was irrigated four days prior to seeding on the 31 October 2002. A Mini Air pneumatic planter was used to plant both the lime-coated and uncoated conditioned seeds (Figure 5.2). The planter was calibrated for seeding rate prior to seeding. Shallow furrows were opened using a stick to plant seed tapes (that included three seeds at 20 cm spacing) and were planted manually. All three seed treatments were planted at two depths, 10 mm and 18 mm. Weed control should pay special attention if guayule need to be established by direct seeding. Foster *et al*. (1999) obtained successful weed control using pre-emergent herbicide, dimethyl 2,3,5,6-tetrachloro-1,4-benzenedicarboxylate (DCPA) at a rate of 4.5 kg ai/ha. Therefore, DCPA was sprayed to control weeds a day after planting at a rate of 4.5 kg ai/ha to control weeds. The field was irrigated daily for the first ten days and then the irrigation interval was gradually reduced to provide sufficient soil moisture until the end of third week, thereafter no irrigation was applied. Precipitation and temperatures (both maximum and minimum) temperatures at the trial site during the study periods were also recorded (Figure 5.3 and 5.4). Data on seedling emergence and survival were recorded at regular intervals up to 52 days and analyzed statistically using Minitab (version 14).

Figure 5.2 Planting of guayule seed using a Mini Air pneumatic planter

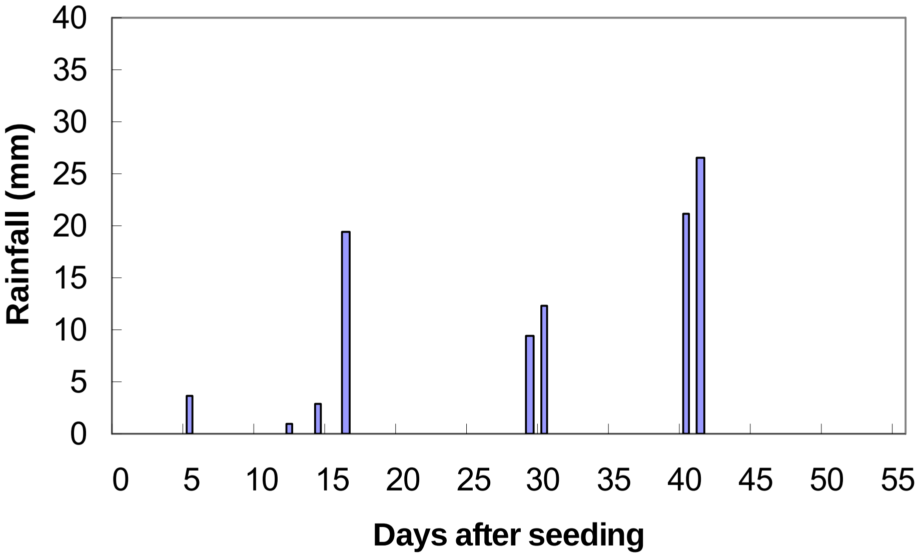


Figure 5.3. Daily rainfall received by the trial site during the study period

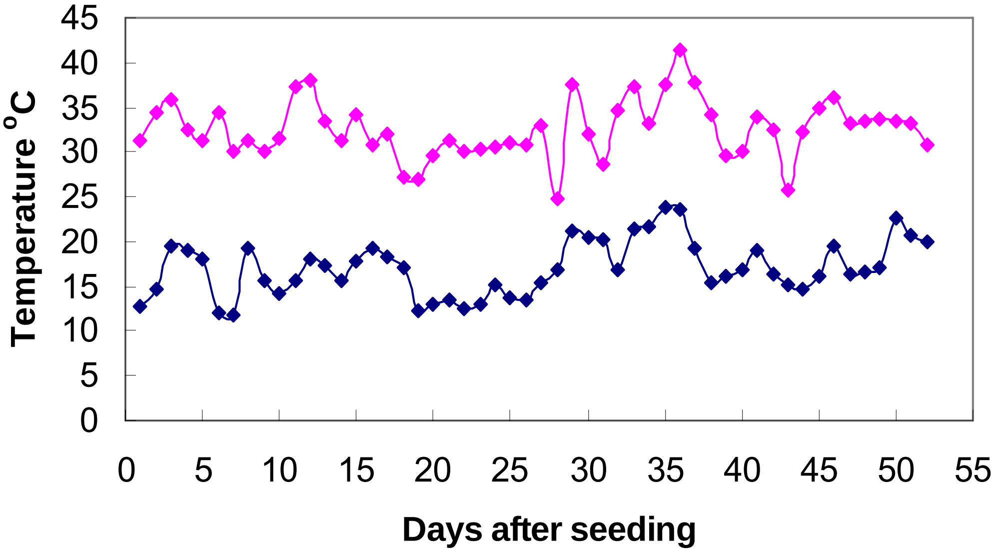


Figure 5.4. Maximum and minimum temperature of the trial site during the study period

5.2.2 Experiment 2

Seed of lines AZ-3 and AZ-5 obtained from the Agricultural Research Service of the USA (ARS-USDA) were used in this experiment. AZ-3 seed was harvested in 1999 and AZ-5 in 2003 (Coffelt, 2003). Seed was separated manually from all foreign matters and then the laboratory seed blower was used to remove unfilled and partially filled seeds. Cleaned seeds were then divided in to three equal lots. One third was left untreated while the second was treated using method of Naqvi and Hanson (1980). The third lot was treated (conditioned) using the method developed by Chandra (1991) using PEG, gibberellic acid, potassium nitrate and thiram. Laboratory germination of each seed lot was performed prior to field planting. The germination test was carried out at constant temperature of 20°C and under 8/16 light and dark conditions. Seed germination was observed from 6 to 14 days. At the end germination counts were recorded for both vigorous seedlings and for poor quality seedlings. Seedlings that had normal shoot and root growth were counted as vigorous seedlings. Seedlings that had abnormal growth, no whole root system or died within 14 days counted as poor quality seedlings.

Land preparation procedure was similar to experiment one. Experimental design used was a randomised complete block with four replicates. Each plot consisted of single row 9 m length, row width was one meter apart. Field sowing was carried out on 27 October 2004 using a Mini Air pneumatic planter. Depth of seeding was maintained at 10 mm. A sprinkler system was used to keep soil moist throughout the first three weeks by irrigating daily whenever rain was not received. Irrigation was stopped after the third week. No herbicides were used and weeding was carried out manually along the row after all seedlings were emerged. Daily rainfall and maximum and minimum temperature throughout the study period were also recorded (Figures 5.5 and 5.6).

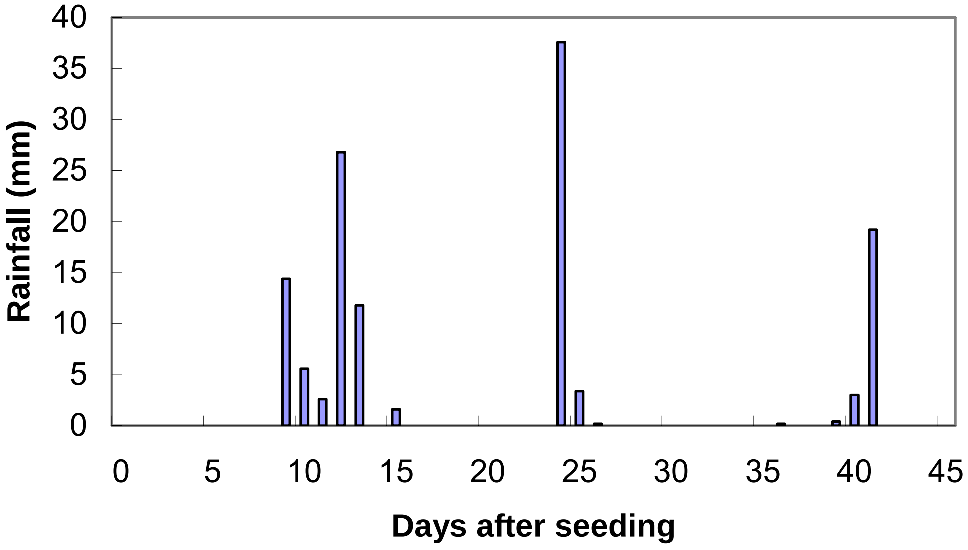


Figure 5.5. Daily rainfall received by the trial site during the study period

Seedling emergence and survival were recorded at regular intervals up to 42 days after planting. Then, seedlings within a randomly selected one-metre length in a row were uprooted to evaluate seedling growth. These seedlings were measured for total plant height (root and shoot), stem and root lengths. Seedlings were then oven-dried at 60°C to measure dry matter production. Data obtained were analysed statistically.

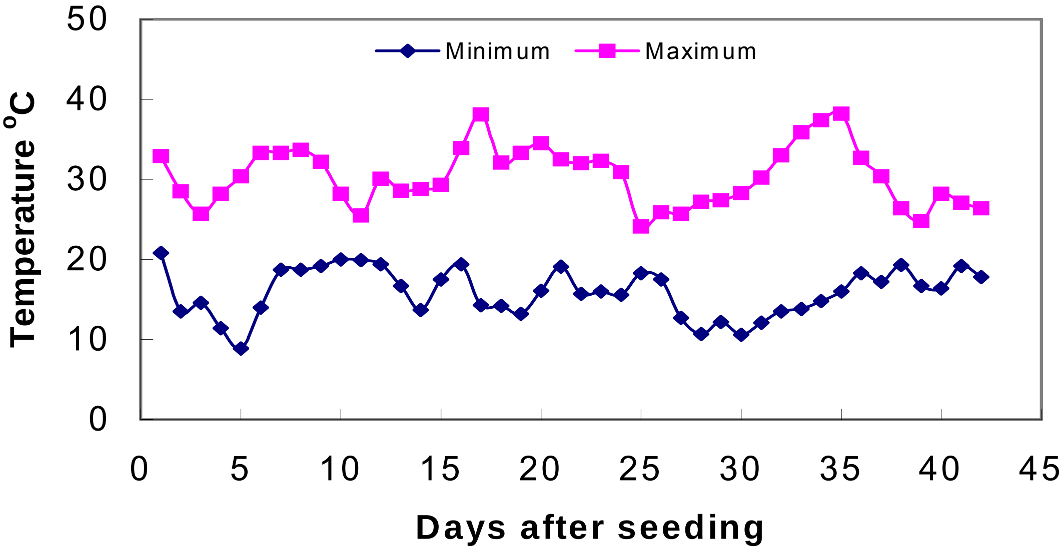


Figure 5.6 Maximum and minimum temperature of the trial site during the study period

5.3 Results

5.3.1 Experiment 1

Seed used in this experiment had low germination percentages ranging from 20% to 27% (Table 5.1). Seedling emergence started 6 days after planting and maximum emergence occurred on the 11th day from planting. Planting depth had a significant effect on seedling emergence (Table

5.2). Seed planted at 10 mm depth produced a mean of 23.2 seedlings per 8 m row and was significantly higher than the 11.9 seedlings from the 18 mm depth planting.

Uncoated, lime coated and taped seed planted at 10 mm depth produced 26.3, 21.0 and 22.4 seedlings/8m respectively. Emergence of these three treatments from total germinable seed planted at 10 mm depth was 25%, 23% and 29% respectively. When they were planted at 18 mm, they only produced an average of 14.8, 10.5 and 10.4 seedling/8m. The low seedling emergence was due to combine effect of low seed germination and low seed rate.

Table 5.1 Laboratory seed germination of different seed treatments and seed rate used at planting

Treatment	**Germination (%)**	**Seed rate (seeds/m)**
Control	27	48
Lime coated	24	47
Seed tape (Paper)	20	15

Manual planting of seed tapes made it difficult to maintain a uniform soil covering and thus might have influenced emergence. Seedling survival dropped dramatically from two weeks to seven weeks (Table 5.3 and Figure 5.7) due to combine effect of low soil moisture and high temperature.

Table 5.2 Effect of seed treatment and planting depth on maximum seedling emergence (by 11 days after planting)

Planting depth (mm)	**Number of seedling/8 m**	**Treatment**	**Seedling emergence**	
			Seedling No./8 m	**Percentage from germinable seeds (survival)**
10	23.2 a	Control	26.3 a	25.2
		Lime coated	21.0 a	23.0
		Seed tape (Paper)	22.4 a**	29.2
18	11.9 b	Control	14.8 a	14.2
		Lime coated	10.5 a	11.5
		Seed tape (Paper)	10.4 a**	13.5

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey’s simultaneous test.

** Corrected for planting rate

Table 5.3. Seedling survival 22 days after planting for treatments at two depths

Treatment	**Planting depth (mm)**	**Number of seedlings in 8 m row**	**Percentage survival of emerged seedlings**
Control	10	14.5 a	55.1
	18	9.5 a	64.2
Lime coated	10	9.5 a	45.2
	18	7.3 a	76.2
Seed tape (Paper)	10	7.2 a**	32.9
	18	8.0 a**	75.8

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

** Corrected for planting rate

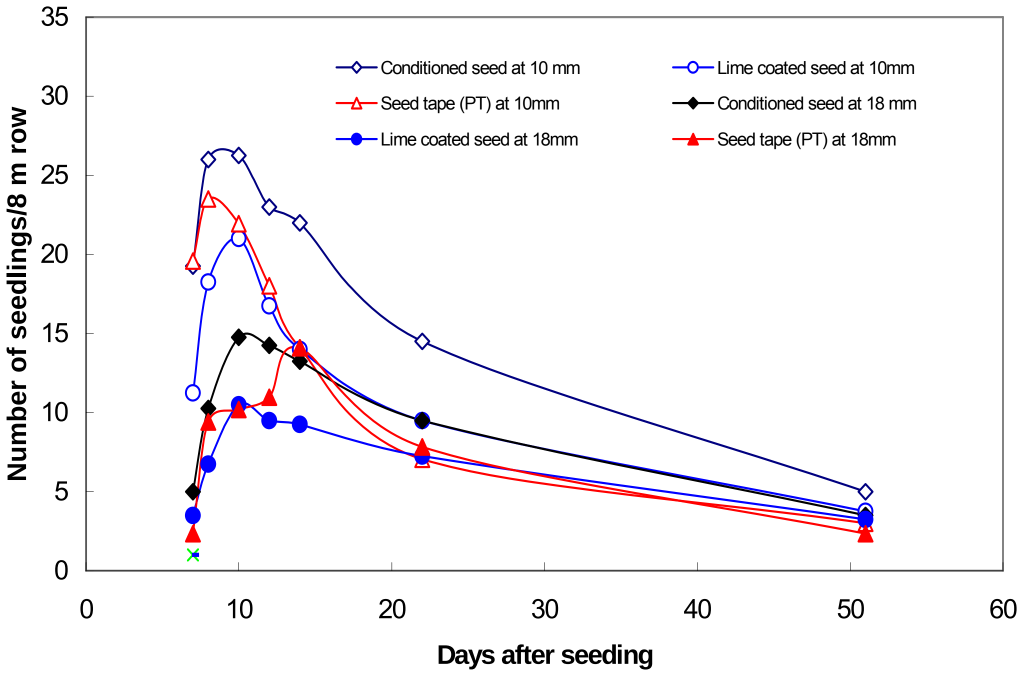


Figure 5.7 Seedling emergence and survival of different seed treatments and planting depth over time

5.3.2 Experiment 2

Seed germination test showed significant differences among treatments (Table 5.4). Germination of both AZ-3 (47.0%) and AZ-5 (45.1%) was significantly higher for conditioned (PEG) seed than untreated controls (35.4% and 35.0% respectively). Increase in germination of conditioned seed of AZ-3 and AZ-5 was 33% and 29% respectively.

AZ-3 had better quality seed with very low level of damage while AZ-5 seeds were poor quality with high levels of damages. Therefore, AZ-5 had only 28.2% vigorous seedlings for conditioned seed and 13.2% for untreated control while almost all seedlings produced by AZ-3 were vigorous. Sodium hypochlorite treatment produced differential effect for the two lines. NaOCl treated AZ-5 seed had significantly higher germination (43.9%) than untreated seed (35.0%). The difference between NaOCl treated AZ-3 seed (38.3%) and untreated seed (35.4%) was not significant.

Table 5.4 Seed germination of treated and untreated seed of lines AZ-3 and AZ-5

	AZ-3		**AZ-5**	
Seed treatment	**Total germination (%)**	**Vigorous seedling (%)**	**Total germination (%)**	**Vigorous seedling (%)**
Control	35.4 b*	35.4 b	35.0 b	13.2 c
NaOCl + GA_3	38.3 b	38.3 b	43.9 a	19.0 b
Conditioned (PEG)	47.0 a	47.0 a	45.1 a	28.2 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Seedling emergence started about 6 - 7 days after planting and maximum emergence was observed in 16 days (Figure 5.8). As germination, AZ-3 and AZ-5 produced variable results for seedling emergence. High levels of seed damage resulted in poor germination and establishment for line AZ-5. Further, seed treatments both conditioning and sodium hypochlorite had a negative effect on seedling emergence for line AZ-5 (Figure 5.8). Because of poor establishment, only seedlings from AZ-3 were used further study on seedling survival and growth.

At maximum emergence, conditioned AZ-3 seed produced 76 seedling/9m that was significantly higher than for untreated seed (41 seedlings/9m) (Table 5.5). The emergence (44 seedlings/9m) from sodium hypochlorite treated AZ-3 seed was not significantly different from the control. Emergence as a percentage of germinable seeds produced by three treatments, untreated, sodium hypochlorite and conditioned seed were 16.8%, 16.7% and 23.2% respectively, these values were not statistically different.

Seedling survival also followed a similar pattern to emergence (Table 5.6). By 42 days after planting, seedlings survival from conditioned seed (67 seedlings/9m) was significantly higher than survival from untreated seed (29 seedlings/9m). Seedling survival of the NaOCl treatment (39 seedlings/9m) was not significantly different from the control. Seedling survival rates of the two seed treatment methods, conditioning and sodium hypochlorite, as a percentage of total emerged seedlings were 89% and 88%, significantly higher than the 70% produced by untreated seeds.

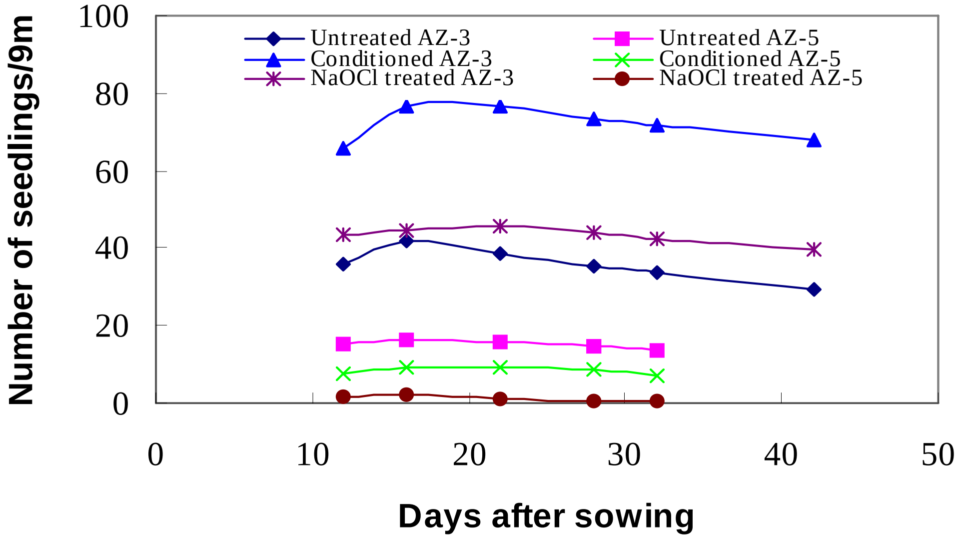


Figure 5.8 Seedling emergence and survival of different seed treatments over time

Table 5.5 Effect of seed treatments on seed germination and seedling emergence

Seed treatment	**Seed rate (seeds/m)**	**Seed germination (%)**	**Maximum seedling emergence (16 days after planting)**	
			Number of seedlings/9 m	**Percentage of germinable seeds (Survival)**
Control	78	35.4 b	41.8 b	16.9 a
NaOCl + GA_3	78	38.3 b	44.8 ab	16.7 a
Conditioned	78	47.0 a	76.5 a	23.3 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Table 5.6. Effect of seed treatments on seedling survival by 42 days after planting

Seed treatment	**Seedling No./9 m**	**Percentage of germinable seeds**	**Percentage survival from emerged seedlings**
Control	29.5 b*	11.9	70.6 b
NaOCl + GA_3	39.5 ab	14.7	88.2 a
Conditioned	67.8 a	20.5	89.1 a

* Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.

Seed conditioning significantly increased seedling growth as indicated by increased root growth (10.1 cm), seedling height (22.4 cm) and seedling dry matter (379 mg/seedlings) compared with the control (8.3 cm, 19.0 cm and 206 mg/seedlings respectively) (Table 5.7, Figures 5.9 and 5.10). Sodium hypochlorite treatments also tended to increase growth compared with the control, however, the differences were not significant.

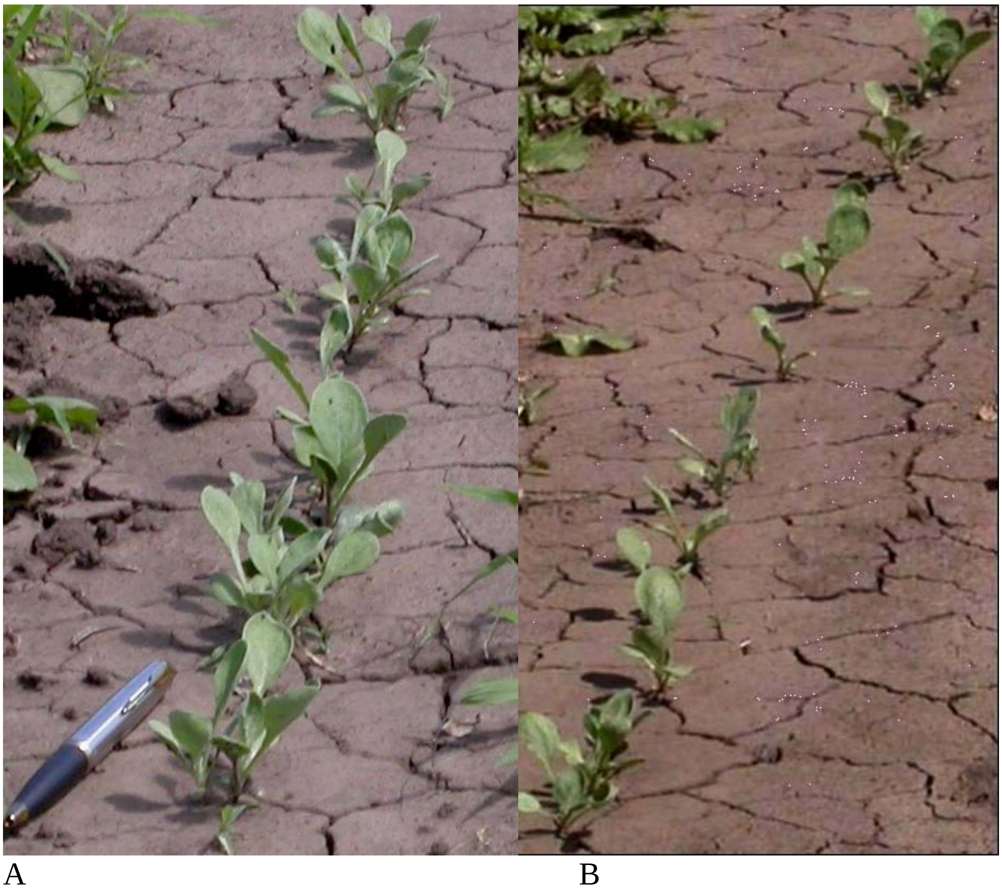
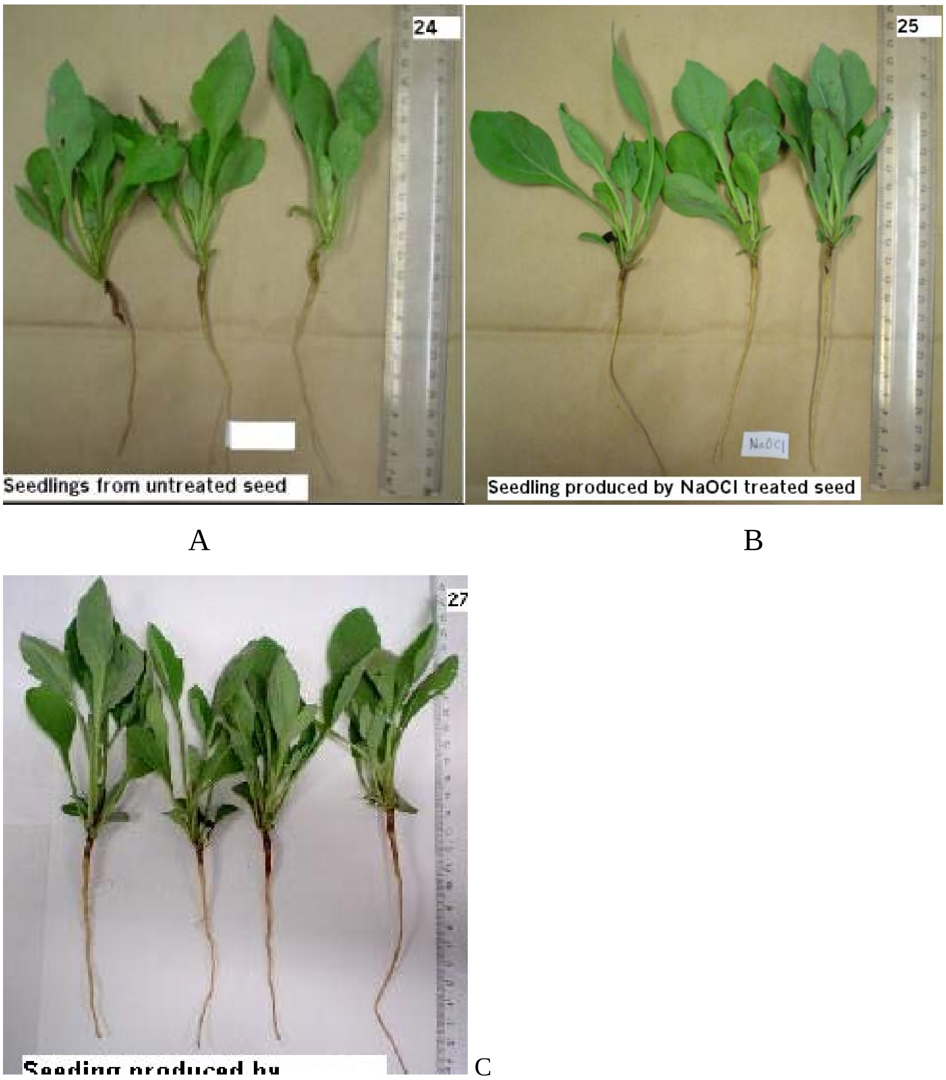


Figure 5.9 Seedling growth of direct seeded guayule four weeks after planting. A Seedlings produced by conditioned seed. B. Seedling of NaOCl and gibberellic acid treated seed.

Table 5.7 Seedling growth and dry matter production of treated and untreated seed of AZ-3 42 days after seeding

Seed treatment	**Stem length (mm)**	**Root length (cm)**	**Plant height (root + shoot) (cm)**	**Dry weight (mg/seedling)**
Control	13.3 a*	8.3 b	19.0 b	206 b
NaOCl + GA_3	13.0 a	8.8 ab	19.1 ab	288 ab
Conditioned (PEG)	13.8 a	10.1 a	22.4 a	379 a

*   Means within a column followed by the same letter are not significantly different at the 0.05 level according to Tukey's simultaneous test.



A B

C

Figure 5.10 Comparison of seedling growth 6 weeks after seeding. Seedlings produced by A. Untreated seeds, B. Sodium hypochlorite and gibberellic acid treated seed and C. Conditioned seed.

Germplasm evaluation trials (Chapter 3) show that new lines produce comparatively fast growth (Figures 3.5, 3.6, 3.14, 3.15). Further seed conditioning has increased seedling vigor (Bucks *et al*. 1986; Foster *et al*. 1999). Results of current study also showed that conditioning improved seedling vigor (Table 5.7 and Figure 5.8). However, growth of guayule is slower than most of the weeds. On the other hand, guayule requires frequent irrigation to establish by direct seeding that aggravates weed problem. Therefore, as mentioned by Foster *et al*. (1999) and Milthorpe (1984) weed control has to be given special attention if guayule is to be established by direct seeding.

5.4 Discussion

Establishment of guayule by direct seeding at the trial site produced encouraging results. Raw seed also produced reasonably good emergence and establishment. However, seed conditioning by polyethylene glycol (PEG) produced much better results than raw seeds or NaOCl plus gibberellic acid treated seeds. Conditioning by PEG not only increased seed germination and establishment but it also increased seedling growth. The results obtained were comparable with findings by Bucks *et al.* (1986), Chandra (1991) and Foster *et al.* (1999).

5.4.1 Germination

In the current study, conditioning increased seed germination by about 33% (from 35.45 to 47%). Chandra and Bucks (1986) obtained increased germination in broad range of temperature by conditioning. Testing 21 lots of 5 to 9 year old seed, Chandra (1991) reported have variable results for conditioning. Two seed lots produced 101% (from 31.8% to 63.8%) and 197% (from 33% to 98%) increase in germination. Six lots had between 14.5% to 37% increased. All other seed lots except 3 had 0% to 10% increased in germination due to seed conditioning.

Foster *et al.* (1999) obtained similar results to the current study with a 35% increase in germination (from 56% to 76%) with conditioned seed. As suggested by Chandra (1991) increased in germination by conditioning could be due to combine effect of gibberellic acid, PEG and KNO_3. In general, seed conditioning appears very effective in improving seed germination for many seeds.

Seed used in the current experiment was about 5 year old (AZ-3) and Chandra (1991) used more than 5 year old seed. Result of both experiments showed that older seed too respond to seed treatments in increasing germination. Further different seed lots produced variable response for same treatment. This support our claim made in Chapter 4 that influence of storage conditions in changing the balance of inhibitors and promoters to impose secondary dormancy.
Sodium hypochlorite together with gibberellic acid seemed to increase seed germination in some seed. The differential effect could be related to the level of dormancy. In general, conditioning is better than NaOCl and gibberellic acid treatment in increasing seed germination.

5.4.2 Emergence

Both experiments one and two in this study produced consistent results for seedling emergence. Conditioned seed in experiment one produced 25% seedlings of the total germinable seeds planted while the value for experiment two was 23%. Untreated seed produced 17% emergence. Therefore, the results of emergence for conditioned seed were consistent for this site. In a laboratory study with several seed lots, Chandra (1991) found that seed conditioning with PEG increased the development of normal seedlings from 1% to 179%. Foster *et al.* (1999) reported to have quicker germination and emergence with conditioned seed compared to raw seed. Bucks *et al.* (1986) also observed increased germination and seedling survival with conditioned seed. Khan *et al.* (1978) reported that number of other species responded to PEG treatment by reducing the time required for germination and seedling emergence and producing uniform establishment. NaOCl and gibberellic acid treatment seemed to have no effect on improving seedling emergence.

It was noticed in the laboratory germination test that many seeds in line AZ-5 were damaged probably during threshing. Lower levels of establishment with AZ-5 compare to AZ-3 in Experiment 2 could be due to the influence of chemicals on damaged seeds. Chemicals especially sodium hypochlorite, potassium nitrate and thiram could have negative effect on damaged seeds. This may be the reason that line AZ-5 produced negative results for treated seeds compared to untreated seeds.

5.4.3 Establishment

Establishment obtained from Experiment 2 (6 weeks after planting) for conditioned seed was 21% of total germinable seed while untreated seed produced only 12%. Increase establishment for conditioned seed was a combined effect of increased germination and vigour. The seedling population of 7.5 seedlings/m achieved by conditioned seed were above the recommended population, 3 seedlings/m. Establishment by untreated seed (3.3 seedling/m) was about the recommended plant population. These results were obtained using a seed rate of 78 seeds/m with seeds having 47% germination. Thus, seed rate needs to be adjusted according to seed germination and the required plant population.

Emergence of experiment 1 was between 25% to 29%. However, seedling survival was poor. This was mainly due to low levels of soil moisture because no irrigation was applied and little rain was received after the third week of planting. Further, low soil moisture combined with high temperature lead to desiccation of seedlings

Foster *et al*. (1999) achieved a high level of establishment 30 days after planting. They established 61 seedlings/m, using conditioned seed with 76% germination planted at a seed rate of 100 seeds/m. Therefore, the establishment was 80% of total germinable seed planted. They also obtained an establishment of 37 seedlings/m using untreated seed with 56% germination planting at same seed rate. Therefore, the establishment was 66% out of total viable seed. This high level of establishment could be due to combined effect of good quality seed together with more favourable soil and environment conditions.

In another study 45% viable seed produced seedlings by three months after planting using conditioned seeds with 33% germination (Bucks *et al*. 1986). They used a light vermiculite cover over the seed and provided good irrigation throughout the study period. These results indicate that establishment by direct seeding produced variable results for variable soil and environmental conditions. NaOCl and gibberellic acid treatment too increased the survival of emerged seedlings. It could be due to the effect of gibberellic acid in improving seedling vigour.

5.4.4 Seedling growth

Seed conditioning was also effective in increasing seedling growth. Conditioned seed produced about 101 mm root growth, 124 mm top growth and dry weight of 379 mg per seedling. Untreated seed only produced 8.3 cm root growth, 11.7 top growth and 206 mg per seedling. Chandra (1991) found PEG significantly increased dry matter (shoot). In a pot experiment, he obtained significantly higher shoot dry matter of 1.6 mg/shoot (for 12 days) by conditioning the seed compared to 1.2 mg/shoot for untreated seed. Therefore, conditioning effectively increased seedling growth.

The combined treatment of NaOCl and gibberellic acid slightly improved the seedling emergence, establishment and growth. However, it was not as effective as seed conditioning.

5.4.5 Lime Coating and seed tape planting

Lime coating of conditioned seed produced slightly lower results for germination and emergence. A slight delay in germination (1 - 2days was noticed for the coating treatment). However seed coating could have a beneficial effect for field performance of direct seeded guayule especially when environmental conditions are not optimum for seed germination and seedling growth.

Our finding in Chapter 4 that gibberellic acid totally replaced the light requirement is useful for future research on seed coating because previously it was believed that coating the seed might reduce germination. But this finding revealed that seed coating can be done without affecting seed germination. In general, seed coating provides several benefits. It protects seed from adverse environmental conditions, protects them from pest attacks and provides required nutrients at a close proximity to the seed. Therefore, the effect of seed coating in improving establishment is worth further investigation.

Seed tape planting was not inferior to coating. It also has benefits of not directly exposing seeds to adverse environmental conditions and pests. However, further studies with variable seed rates and mechanized planting are required.

5.4.6 Requirements in direct seeding

As stated by Whitworth (1981) and Foster *et al.* (1999), there are several requirements to achieve better establishment by direct seeding. They are:

- High quality seed with high levels of purity is a basic requirement to achieve good establishment.
- Precision planting is also an important aspect to planting seed at a shallow depth of about 10 mm without affecting seedling emergence. Therefore, land preparation is an important factor in obtaining a fine textured seedbed with a uniform surface.
- Soil moisture within the first three to four week of planting is very important. In this study, lack of enough soil moisture resulted in very low establishment in Experiment 1 whereas adequate soil moisture in Experiment 2 produced better results. Therefore, this supports the research by Foster *et al.* (1999) that frequent irrigation during the first 30 days is important to promote seed germination, prevent soil crusting to facilitate seedling emergence and to protect young seedlings against desiccation. However, excessive soil moisture that can cause damping off diseases associated with soil borne fungi, *Pythium* sp., *Phytophthora* sp., *Fusarium* sp. and *Rhizoctonia* sp., should be avoided (Mihail *et al*. 1991).
- Guayule seedlings grow slowly, producing only 10 mm top growth and 50 mm root growth 2 weeks after emergence (Miyamoto and Bucks 1985). Seedlings in this study produced 124 mm top growth and 101 mm root growth 6 weeks after planting. Therefore, guayule seedlings cannot compete effectively against weeds. For this reason, effective pre-emergent herbicides need to be introduced if guayule is to be direct seeded for commercial plantings (Foster *et al*. 1993). Successful weed control was achieved by using pre-emergent herbicide of DCPA at a rate of 4.5 to 9.0 kg ai/ha depending on soil type (Foster *et al*. 1993).

5.4.7 Potential in Queensland

Direct seeding of dryland sites in Queensland presents several challenges. Initially the seedbed needs to be well prepared to maximize establishment of the small seeded guayule. As indicated, the seed must be planted at a shallow depth (not greater than 10 mm). This then exposes the seed and seedlings to desiccation. The optimum situation would be to plant into a moist seedbed and for conditions following planting to be mild. In the present study, plantings occurred in late October and maximum temperatures greater than 30°C were relatively common thereafter. Early plantings may reduce potential desiccation by allowing germination seedling development to occur under milder temperatures. Autumn planting would be another option to avoid exposure of high temperature for emerging seedlings and young seedlings at early stages of growth. Once established, guayule can tolerate lower temperatures during winter. Therefore, early to mid autumn would be the ideal time for planting. This will establish seedlings before temperatures are too cold. An alternative approach would be fluid drilling whereby seed is planted in a viscous medium that aids germination and seedling development during the critical early stages.

6. Implications and recommendations

Germplasm evaluation

This research has identified suitable lines for guayule production in south-east Queensland. These lines have greatly improved rubber and resin yields due to improved biomass compared with old lines. Improvement in both these attributes will increase the economic viability of the crop. Australia has large areas of land with soils and climate suitable to guayule production. Yulex Corporation (U.S. based) has recently expressed interest in setting up a pilot plant in Australia to process the crop. There is thus considerable opportunity for import replacement from guayule production; however, the major use for guayule rubber may come from high end medical uses which need a low-allergenic, high quality source.

Overall performance of plant growth, dry matter, rubber and resin yields of new guayule lines, AZ-1 to AZ-6, at two different sites in Queensland, Australia was generally better than that of the old lines, N 565 and 11591.

AZ-1 and AZ-2 were the two best guayule lines for both environmental conditions. They maintained high levels of uniformity and produced early vigorous growth, increased dry matter, increased rubber and resin yields. They also can be harvested in two years under favourable soil and climatic conditions. AZ-2 was preferred to AZ-1 due to its comparatively high uniformity. The yield improvement of these lines would improve the profitability of the crop in Australia.

AZ-1 and AZ-2 produced rubber yields of 567 kg/ha and 611 kg/ha respectively in the second year (17 months) at Gatton from stem and branches (48% to 61% higher yield than the old lines). These two lines produced rubber yields of 717 kg/ha and 787 kg/ha respectively from stem and branches in the third year (33 months) at Chinchilla. Thus the yield increase over the old lines was between 86% and 107%.

AZ-5 and AZ-6 also have favourable features of high rubber content. They produced increased rubber yields after three years of growth. However, uniformity of these lines is lower than AZ-1 and AZ-2 and both lines require further selection. At Gatton, AZ-5 produced the highest rubber yields of 565 kg/ha and 966 kg/ha from stem and branches after the second and third years respectively. This is a 43% increase in yield over the old lines. However, AZ-5 did not outperform the other new lines at Chinchilla.

AZ-6 had the second best rubber yield of 855 kg/ha in the third year at Gatton, that is 27% to 38% higher than the old lines. It also produced the third best yield of 668 kg/ha at Chinchilla, that is 74% to 76% increased in rubber yield over old lines. However, it produced highly variable plant growth at Chinchilla.

A note of caution is needed regarding soil type. Guayule appears to perform well on slow draining cracking clay soils (as occur at Gatton) until waterlogging occurs. This can result in rapid plant death and may necessitate planting of the crop on well drained soils such as sandy loams.

Seed dormancy

Seed germination experiments revealed that dormancy or germination in guayule was regulated by the balance of growth promoters and inhibitors. Dormancy and germination of guayule seed depend upon the corresponding shift in the threshold levels of growth promoters and inhibitors. Endogenous or exogenous growth regulators can shift the balance to inhibit or induce germination. Further, the level of dormancy or rate and extent of germination depends upon the degree of shift from the threshold levels.

High levels of dormancy with freshly harvested seed are due to high levels of inhibitor, abscissic acid (ABA) and/or phenolic compounds, and/or low levels of promoter, gibberellic acid (GA). Environmental conditions during seed development on the mother plant determine the levels of growth regulators (GA and ABA) and hence the dormancy. Therefore extreme environmental conditions produce seed with high levels of dormancy.

The seed coat also contains inhibitors that affect the balance between promoter and inhibitor. Therefore, gibberellins are required to overcome germination constraints imposed by the seed coat and ABA-related embryo dormancy. The seed coat also acts as mechanical barrier to the emerging radicle. Therefore, removing the seed coat improves germination.

Light requirement or response to gibberellin in germination of guayule is mediated through phytochrome. Therefore, light and its quality are also important to activate phytochrome to induce the synthesis of gibberellins and thus promote germination. Further, the light requirement in germination can totally be replaced with exogenous gibberellic acid. However, the required amount or concentration varies according to the level of dormancy.

Direct seeding

Establishment of guayule by direct seeding is possible. However, high quality seed, fine textured uniform seedbeds, shallow precision planting, adequate soil moisture throughout first three weeks and efficient weed control method is required to achieve good establishment by direct seeding. This enables the cost of establishing the crop to be greatly reduced compared with transplants thus increasing profitability.

This study confirmed that osmoconditioning with polyethylene glycol, as developed by Chandra and Bucks (1986), is very effective in increasing germination, emergence and establishment. It also improves seedling growth at the initial stage.

Recommendations for further studies

Microclimates within the regions suitable for guayule could affect plant growth, dry matter and rubber yield in different ways to those observed in the present studies. Therefore, further evaluation trials using the best performing lines (AZ-1 and AZ-2) will be helpful. These trials need to be carried out on different soil types and under irrigated and dryland conditions within the recommended regions.

All lines except AZ-2 and AZ-3 had different percentages of off-types. These lines especially AZ-4, AZ-5 and AZ-6 have high rubber contents compared to other new lines. However, for these lines to become commercial, the high percentage of off-types needs to be reduced because it affects the final rubber yield. To this end, further plant selection for uniformity, biomass and rubber content is required to

Ratoon crops are possible with guayule. Root data was obtained from the Chinchilla trial but the duration of the project was not sufficient to obtain ratoon yields for 3 -5 year old crops. Also, ratooning ability may vary with different lines. Studies are needed to evaluate the performance and economics of ratoon crops.

Available data on plant population studies is based on old low yielding lines. New lines produce large canopies with faster growth. Therefore, plant population studies need to be carried out with these new high yielding and fast growing lines to determine optimum plant populations. These populations are likely to vary with soil moisture status.

Root diseases are the only disease condition that caused significant threat to guayule. It could pose severe problems in the future commercial guayule plantings. Further research is being conducted at the University of Queensland to investigate this aspect. Selection of lines resistant to root rot may be highly beneficial for commercial production of guayule.

The current study has furthered understanding of mechanisms of seed dormancy in guayule. These findings have enabled the development of a cost effective and efficient seed treatment to improve germination and establishment of guayule.

Direct seeding studies here as well as in the USA have produced encouraging results. These studies have been conducted under irrigated conditions and highlight the need for well prepared and controlled seedbed environment. However, irrigation in semiarid regions is often not available. Therefore, direct seeding studies under these conditions is necessary if the crop is to be successfully established. A number of strategies need to be investigated:

- fluid drilling
- seed coat technology
- time of planting to avoid high summer temperature during early seedling growth (early spring or autumn sowings)
- cultural practices such as surface mulching to minimise soil

High quality seed is very important especially for direct seeding of guayule. More efficient harvesting and processing equipment is required. Optimising harvesting of seed is a challenge due to prolonged flowering over summer and shattering. Processing is also challenging due to the small seed size (1mg) and the difficulty of separating light and empty seeds from filled seeds. Presently, research on harvesting and processing of guayule is underway at the University of Queensland. The influence of plant population on seed yield and size is also being investigated. Large seed size may improve establishment by direct seeding.

7. Bibliography

Ahlawat, A.S., Dagar, J.C. and Singh, V.P. (1979). Seed germination studies on *Parthenium hysterophorus* Linn. Proceedings of the Indian National Science Academy-B, 45(6): 613-616.

Akers, S.W. and Holley, K.E. (1986). A system for priming seeds using aerated polyethylene glycol or salt solutions. HortSci., 21: 529-531.

Akers, S.W. (1990). Seed response to priming in aerated solutions. Search, 19: 8-17.

Allen, S.G., Nakayama, F.S., Dierig, D.A. and Rasnick, B.A. (1987). Plant water relations, photosynthesis, and rubber content of young guayule plants during water stress. *Agron. J.* 79: 1030-1035

Amen, R.D. (1968). A model of seed dormancy. Bot. Rev., 34:1-31.

Anon., (1977), Guayule an alternative source of natural rubber, National Academy of Sciences, Washington, D.C. 80p.

Anon., (1980). Ecology and reproduction of guayule (*Parthenium argentatum* Gray) on natural stands in Cahuila, Neuvohesn and Zacatecas, Mexico. Native Plant Inc. A report presented for CIQA (Cetro de Investigacion en Quimica Aplicada), Saltillo, Mexico.

Anon., (2000). New Industrial Crop Research, USDA-ARS, Available at: http://www.uswcl.ars.ag.gov/epd/newcrops/newcrops.htm

Archer, B.L. and Audley, B.G. (1973). Rubber, gutta percha and chicle. In: Nord, F.F. and Miller, L.P. (eds.) Phytochemistry. 2: 310-343.

Artschwager, E. (1943). Contribution to the morphology and anatomy of guayule (*Parthenium argentatum* Gray). Technical Bulletin, U.S. Dep. of Agriculture. 34 p.

Auchter, J., Kaelin, T. and Kishi, A. (2000), Chemical Economics Handbook: Natural Rubber.

Backhaus, R.A. and Walsh, S. (1983). The ontogeny of rubber formation in guayule, *Parthenium argentatum*. Bot. Gaz., 144(3); 391-400.

Backhaus, R.A. (1985). Rubber formation in plants – a mini-review. Israel J. Bot. 34: 283-293.

Backhaus, R.A., Higgins, R.R. and Dierig, D.A. (1989). Photoperiodic induction of flowering in guayule. HortSci., 24(6): 939-941.

Batak, I., Devic, M., Giba, Z., Grubisic, D.,Proff, K.L. and Konjevic, R. (2002). The effect of potassium nitrate and NO-donors on phytochrome A- and phytochrome B- specific induced germination of *Arabidopsis thaliana* seeds. Seed Science Research. 12; 253-259.

Bealing, F.J. (1969). Carbohydrate metabolism in *Hevea* latex – availability and utilization of substrates. J. Rubber Res. Inst. Malays. 21: 445-455.

Beckett, R.P., Van Staden, J. and Shirley, A., (1992). The effect of micronutrient supply on the growth and seed production of guayule (*Parthenium argentatum* Gray). Ann. Appl. Biol., 120; 567-571.

Benedict, H.M. and Robinson, J. (1946). Studies on the germination of guayule seed. Washington, U.S. Dept. of Agriculture, 1946. 48 p.

Benedict, H.M. (1949). A further study on the nonutilization of rubber as a food reserve by guayule. Bot. Gaz., Vol. 111(1): 36-43.

Benvenuti, S. and Lercari, B. (1994). Effect of canopy light environment during seed ripening on germination of *Daura stramonium* L. seeds. Agric. Mediterr. 124: 86-91.

Bewley, J.D. and Black, M. (1994). Seeds physiology of development and germination, 2nd Ed. Plenum Press, New York, USA.

Bonner, J. (1943). Effect of temperature on rubber accumulation by the in guayule plant. Bot. Gaz., 105(2): 233-243.

Bonner, J. (1944). Effect of varying nutritional treatments on growth and rubber accumulation in guayule. Bot. Gaz., 105(3): 352-364.

Bonner, J. and Galston, A.W. (1947). The physiology and biochemistry of rubber formation in plants. Bot. Rev., 13:543-596.

Bonner, J. (1991). The history of rubber. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 1-16.

Bourgoin, Aand Simpson, J.D. (2004). Soaking, moist-chilling, and temperature effects on germination of Acer pensylvanicum seeds. Canadian-Journal-of-Forest-Research. 34(10): 2181-2185.

Bradford, K.J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortSci. 21: 1105-1112. 351-396.

Bradford, K.J. (1995). Water relations in seed germination, In: Development and Germination (Eds.) Kigel, J. and Galili, G. Marcel Dekker, New York, USA.

Bucks, D.A., Roth, R.L., Nakayama, F.S. and Gardner, B.R. (1985). Irrigation water, nitrogen, and bioregulation for guayule production. Trans. ASAE. 28(4): 1196-1205.

Bucks, D.A., Roth, R.L., Powers, D.E. and Chandra, G.R. (1986). Direct seeding for economical guayule field establishment. In: Fangmeier, D.D. and Alcorn, S.M. (Eds.), Guayule, a Natural Rubber Source. p 77-87.

Burritt, D.J. and Hunter D.C. (2004), Light quality influences adventitious shoot production from cotyledon explants of lettuce (*lactuca sativa* l.), In Vitro Cellular and Development Biology - Plant, 40(2); 215-220.

Chandra, G.R. and Bucks, D.A. (1986). Improved quality of chemically treated guayule (*Parthenium argentatum* Gray) seeds. In: Fangmeier, D.D. and Alcorn, S.M. (Eds.), Guayule, a Natural Rubber Source.: 59-68.

Chandra, G.R. (1991). Osmo-conditioning improves guayule seed quality. Bioresource Technol. 35(2): 141-145.

Chong, C., Bible, B.B. and Ju, H.Y. (2002). Germination and emergence. In: Pessarakli, M. (Ed.). Handbook of plant and crop physiology. Marcel Dekker, Inc. New York, USA.

Coates, W. (1985). Development of a guayule seed harvester. Trans. ASAE. 28(3): 687-690.

Coates, W. (1990). Equipment for harvesting guayule seeds and shrubs. Proceedings of the Conference on Agricultural Engineering. The Institute of Engineers Australia.

Coates, W. (1991). Guayule harvesting equipment. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 241-259.

Copeland, L.O. and McDonald, M.B. (2001). Principles of seed science and technology, 4th Ed., Kluwer Academic Publishers, USA.

Cornish, K., Pan, Z. and Backhaus, R.A. (1993). Engineering new domestic sources of natural rubber. 192-196. In: Janick, J. and Simon, J.E. (Eds.). New Crops. Wiley, New York.

Cornish, K. and Siler, D.J., (1996). Hypoallergenic guayule latex: research to commercialization. In: Janick, J. (Ed.), Progress in new crops. Proceedings of the Third National Symposium, Oct. 22-25, 1995, Indianapolis, Indiana, VA. pp. 327-335.

Cornish 1996 Patent doc

Cornish, K., (2001). Similarities and differences in rubber biochemistry among plant species. Phytochem., 57, 1123-1134.

Cornish, K. and Backhaus, R.A. (2003). Induction of rubber transferase activity in guayule (*Parthenium argentatum* Gray) by low temperatures. Ind. Crops Prod. 17(2) 83-92.

Cresswell, E.G. and Grime, J.P. (1981). Induction of a light requirement during seed development and its ecological consequences. Nature. 291: 583-585.

Crocker, R.L. and Trumble, H.C. (1945). Investigations guayule (*Parthenium argentatum* Gray) in South Australia. CSIR Bulletin no. 192, Melbourne, Australia.

Currah, I.E., Gray, D. and Thomas, T.H. (1974). The sowing of germinating vegetable seeds using a fluid drill. Ann. Appl. Biol. 76: 311-318.

Curtis, O.F., Jr. (1947). Distribution of rubber and resins in guayule. Plant Physiol., 22: 333-359.

Darby, R.J. and Salter, P.J. (1976). A technique for osmotically pre-treating and germinating quantities of small seeds. Ann. Appl. Biol. 83: 313-315.

Debeanjon, I. And Koornneef, M. (2000). Gibberellin requirement for arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiology. 122: 415-424.

Da Cruz, M. (2003) Studies on the germination and physical properties of Guayule (*Parthenium argentatum* Gray) seed.

Dierig, D.A., Ray, D.T., Coffelt, T.A., Nakayama, F., Leake, G.S. and Lorenz, G. (2001). Heritability of height, width, resin, rubber, and latex in guayule (*Parthenium argentatum*). Ind. Crops Prod., 13, 229-238.

Ehrler, W.L. and Nakayama, F.S. (1984). Water stress status in guayule as measured by relative leaf water content. Crop Sci., 24(1): 61-66.

Emparan, P.R. and Tysdal., H.M. (1957). The effect of light and other factors on breaking the dormancy of guayule seed. Agron. J. 15-19.

Ester, A., Vogel, R. de and Bouma, E. (1997). Controlling thrips tabaci (Lind.) in leek by film-coating seeds with insecticides. Crop Protec. 16(7): 673-677.

Estilai, A. (1991). Biomass, rubber, and resin yield potentials of new guayule germplasm. Bioresource Technol., 35, 119-125.

Estilai, A. and Ray, D.T. (1991). Genetics, cytogenetics, and breeding of guayule. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 47-92.

Evenari, M. (1965). Light and seed dormancy. Encyclopedia of Plant Physiology. 15: 805-847.

Fangmeier, D.D., Rubis, D.D., Taylor, B.B. and Foster, K.E. (1984). Guayule for rubber production in Arizona. Ariz. Agric. Exp. Sta. Tech. Bull. 252.

Fenner, M. (1991). The effect of the parent environment on seed germinability. *Seed Sci. Res*., 1, 75-84.

Ferraris, R., (1986). Agronomic practices for the production of guayule. In: Stewart, G.A. and Lucas, S.M. (Eds.). Potential production of natural rubber from guayule (*Parthenium argentatum*) in Australia. CSIRO, Australia, pp. 97-110.

Ferraris, R. (1993). Effect of plant density on yield and rubber accumulation in guayule (Parthenium argentatum) in south-eastern Queensland. Aust. J.Exp.Agric., 33, 71-82

Foster, M.A. and Moore, J. (1992). Direct-seeding techniques for guayule stand establishment in west Texas. J. Prod. Agric., 5(1): 163-167.

Foster, M.A., Kleine, L.G. and Moore, J. (1993). Response of direct-seeded guayule to pre-emergence herbicides. J. Am. Oil Chem. Soc., 70(12): 1239-1240.

Foster, M.A., Fowler, J.L. Kleine, L.G. and Puppala, N. (1999). Establishing new guayule lines by direct seeding. Ind. Crop. Prod., 9(2): 93-100.

Foster, M.A., Fowler, J.L. Kleine, L.G., Grote, M.M. and Puppala, N. (2001). Performance of direct seeded and transplanted guayule. Ind. Crop. Prod., 15: 23-31.

Frankland, B. and Tayloson, R. (1983). Light control of seed germination; in Shropshire, W. and Mohr, H. (Eds.) *Photomorphogenesis*, 16A, 428-456.

Frischknicht, O., Thielebein, M. and Grahl, A. (1961). Sekundare Keimruhe bei Getreide. Proc. Int. Seed Testing Assoc., 26: 89-114.

Furutani, C.S. Zandstra, B.H. and Price, H.C. (1985). Low temperature germination of celery seeds for fluid drilling. J. Am. Soc. Hort. Sci. 110: 153-156.

Gartside, G., (1986). Guayule processing, rubber quality and possible byproducts, In: Stewart, G.A. and Lucas, S.M. (Eds.). Potential production of natural rubber from guayule (*Parthenium argentatum*) in Australia. CSIRO, Australia, pp. 111-132.

Gorski, T. (1975). Germination of seeds in the shadow of plants. *Physiologia Plantarum*, 34, 342-346.

Goss, R. (1991). The morphology, anatomy, and ultrastructure of guayule. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 33-45.

Grappin, P., Bouinot, D., Sotta, B., Miginiac, E. and Jullien, M. (2000). Control of seed dormancy in *Nicotiana plumbaginifolia*: post-imbibition abscisic acid synthesis imposed dormancy. Planta, 210(2): 279-285

Greipsson, S. (1999). Seed coating improves establishment of surface seeded *Poa pratensis* used in revegetation. Seed Sci. Technol. 27(3): 1029-1032.

Groves, R.H., Hahon, M.W. and Ramakrishnan, P.S. (1982). Dormancy and germination of seed of eight populations of *Themeda australis. Aust. J. Bot.,* 30, 373-386.

Guedes, A.C. and Cantliffe, D.J. (1980). Germination of lettuce (*Lactuca sativa*) at high temperature after seed priming. J. Am. Soc. Hort. Sci., 105: 777-781.

Hammond, B.L. (1959). Effect of Gibberellin, Sodium Hypochlorite, Light, and Planting Depth on Germination of Guayule Seed. Agron. J., 51(10): 621-623.

Hammond, B.L. and Polhamus, L.G. (1965). Research on guayule (*Parthenium argentatum*), 1942-1959. U.S. Dept. of Agriculture, 157 p.

Heide, O.M. and Prestrud, A.K. (2005). Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree-Physiology. 25(1): 109-114.

Henderson, K, (1983). Observations on current guayule research and development in the United States and Mexico: implications for Australia. CSIRO Division of Water and Land Resources. Tech. Memo. 83/1. Canberra, Australia.

Hendricks, S. and Taylorson, R. (1972). Nature, 237: 167.

Hopkins, W.G. (1999). Introduction to plant physiology. 2nd ed. John Wiley & Sons, New York, USA.

Hsiao , A.I. and Quick, A.W. (1984). Actions of sodium hypochlorite and hydrogen peroxcide on seed dormancy and germination of wild oats, *Avena fatua* L. Weed Research. 24: 411-419.

Huang, H.T., (1991). Introduction, In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, p. xv.

Hunter, A.S. and Kelley, O.J. (1946). The growth and rubber content of guayule as affected by variations in soil moisture stresses. J. Am. Soc. Agron., 38(2): 118-134.

Islam, M.O., Matsui, S.and Ichihashi, S. (2000). Effects of light quality on seed germination and seedling growth of *Cattleya orchids* in vitro. Journal of the Japanese Society for Horticultural Science. (abstract available in CAB Abstract 1999; 68(6):

Ji, W, Chauncey, R and Foster, M.A. (1993). Seasonal variation in rubber biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, and rubber trasferase activities in *Parthenium argentatum* in the Chihuahuan deseart. Plant Physiol., 103: 535-542.

Khan, A.A., Tao, K.L., Knypl, J.S., Borkowska, B. and Powell, L.E. (1978). Osmotic conditioning of seeds: Physiological and Biochemical Changes. Acta Horticulturae: 83, 267-278.

Khan, A.A., Peck, N.H. and Samimy, C. (1980/1981). Seed osmoconditioning: Physiological and biochemical changes. Isr. J. Bot. 29: 133-144.

Khan, A.A. (1992). Preplant physiological seed conditioning. Hort. Rev. 13: 131-181.

Kigel, J., Ofir, M. and Koller, D. (1977). Control of the germination responses of *Amaranthus retroflexus* L. seeds by their parental photothermal environment. *J. Exp. Bot.*, 28(106), 1125-1136.

Kubik, K.K., Eastin, J.A., Eastin, J.D. and Eskridge, K.M. (1988). Solid matrix priming of tomato and pepper. Proc. Int. Conf. Stand Est. Hortc. Crops, Lancaster, PA. pp 86-96.

Kuruvadi, S., Rodríguez, M.E.A. and López-Benítez, A., (1997a). Guayule ploidy levels in a native population, Ind. Crops Prod., 6, 83-87

Kuruvadi, S., Jasso Cantu, D. and Angulo-Sanchez, J.L., (1997b). Rubber content in different plant parts and tissues of Mexican guayule shrubs, Ind. Crops Prod., 7, 19-25

Langan, T.D., Pendleton, J.W. and Oplinger, E.S. (1986). Peroxide coated seed emergence in water-saturated soil. Agron. J. 78: 769-772.

Leubner-Metzger. (2001). Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. Planta. 213: 758-763.

Lester, R.N. (1985). Inheritance of hard seed in cotton. Crop Sci., 15: 149-154.

Lloyd, F.E. (1911). Guayule (*Parthenium argentatum*), a rubber plant of the Chihuahuan desert. Carnegie Inst. Washinton. Carnegie Institute Publication no. 139, Washington.

Maher, J.M. (Ed.) (1996). Understanding and managing soils in the Murilla, Tara and Cinchilla shires, Department of Primary Industries Training Series QE96001, Brisbane, Australia.

Mayer, A.M. and Poljakoff-Mayber, A. (1989). The germination of seeds. New York, Pergamon Press. USA.

McCallum, W.B. (1941). The cultivation of guayule. India Rubber World. 105: 33-36.

McDonald, M.B. (2000). Seed priming. In: Black, M. and Bewley, J.D. (Eds.). Seed technology and its biological basis. pp 287-325. Sheffield Academic Press, UK.

Mehta, I.J., Dhillon, S.P. and Hanson, G.P. (1979). Trichome morphology as an indicator of high rubber bearing guayule (*Parthenium argentatum* Gray) plants in native populations. Amer. J. Bot., 66(7); 796-804.

Mexal, J., Fisher, J.T., osteryoung, J. and Reid, C.P. (1975). Oxygen availability in polyethylene glycol solutions and its implications in plant-water relations. Plant Physiology. 55: 20-24.

Michel, B.E. and Kaufmann, M.R. (1973). The osmotic potential of polyethelene glycol 6000. Plant Physiology, 51: 914-916.

Mihail, J.D., Alcorn, S.M. and Whitworth, J.W. (1991). Plant health: The interaction of guayule, micro-organisms, arthropods and weeds. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, 145-172.

Milthorpe, P.L. (1984). Guayule research and development in New South Wales 1980-1983. New South Wales, Department of Agriculture, 1984. 45 p.

Milthorpe, P.L., Sanderson, G.P. and Nicol, H.I., (1994). Effect of plant density on yield, rubber and resin accumulation in guayule in central-western New South Wales. Ind. Crops Prod., 3, 151-163.

Mitchell, J.W., Benedict, H.M. and Whiting, A.G. (1944). Growth, Rubber Storage, and Seed Production by Guayule as Affected by Boron Supply. Bot. Gaz., 106(2): 148-157.

Miyamoto, S., Piela, K., Davis, J. and Fenn, L.B. (1984). Salt effects on emergence and seedling mortality of guayule. Agron. J., 76(2): 295-300.

Miyamoto, S.,K. Piela, K. Petticrew, J. (1985). Salt effects on germination and seedling emergence of several vegetable crops and guayule. Irrig. Sci., 6(3): 159-170.

Miyamoto, S. and Bucks, D.A. (1985). Water quantity and quality requirements of guayule: current assessment. Agric Water Manag., 10, 205-219.

Mooibroek, H. & Cornish, K., (2000). Alternative sources of natural rubber. App. Microbiol. Biotech., 53, 355-365

Muller, C.H. (1946). Root development and ecological relations of guayule. Washington, U.S. Dept. of Agriculture, 1946. 114 p.

Nakayama, F.S. (1991). Influence of environment and management practices on rubber quality and quantity. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 217-240.

Nakayama, F.S., Bucks, D.A., Gonzalez, C.L. and Foster, M.A. (1991a). Water and nutrient requirements of guayule. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 145-172.

Nakayama, F.S., Bucks, D.A., Roth, R.L. and Gardner, B.R., (1991b). Guayule biomass production under irrigation, Bioresource Technol., 35, 173-178

Nakayama, F.S., Vinyard, S.H., Chow, P., Bajwa, D.S., Youngquist, J.A., Muehl, J.H. and Krzysik. A.M., (2001). Guayule as a wood preservative, Ind. Crops Prod., 14, 105-111

Naqvi, H.H. and Hanson, G.P. (1980). Recent advances in guayule seed germination procedures. Crop Sci., 20(4): 501-504.

Naqvi, H.H. and Hanson, G.P. (1982). Germination and growth inhibitors in guayule (*Parthenium argentatum* Gray) chaff and their possible influence in seed dormancy. Am. J. Bot., 69(6): 985-989.

Ni, B.R. and Bradford, K.J. (2004). Germination and dormancy of abscisic acid- and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds (sensitivity of germination to abscisic acid, gibberellin, and water potential). *Plant Physiol.*, 101(2); 607-617.

Nix, H.A. (1986). Land evaluation for potential guayule rubber production in Australia. In: Stewart, G.A. and Lucas, S.M. (Eds.). Potential production of natural rubber from guayule (*Parthenium argentatum*) in Australia. CSIRO, Australia, pp. 27-58

Ojeda, H. and Trione, S.O. (1990). Effect of high temperatures on germination of guayule seeds. Seed Sci. & Technol., 18: 681-691.

Peters, N.C.B. (1982). Production and dormancy of wild oat seed from plants grown under soil water stress. *Ann. Appl. Biol.*, 100, 189-196.

Powell, B. (1982). Soils of the Gatton Research Station, Queensland Depatment of Primary Industries.

Rawlins, W.H.M. (1986). Guayule economics. In: Stewart, G.A. and Lucas, S.M. (Eds.). Potential production of natural rubber from guayule (*Parthenium argentatum*) in Australia. CSIRO, Australia, pp. 133-150.

Ray, D.T. and Janick, J., (1993). Guayule: a source of natural rubber. New crops 338- 342, Proceedings of the Second National Symposium on New Crops: Research and Commercialization, held in Indianapolis, IN, USA, on 6-9 October 1991.

Ray, D.T. Dierig, D.A., Thompson, A.E. and Coflet, T.A., (1999). Registration of six guayule germplasms with high yielding ability. Crop Sci., 39, 300.

Retzer, J.L. and Mogen, C.A. (1947). Soil-guayule relationships. J Am Soc Agron 39(6): 483-512.

Rolston, M.P. (1978). Water impermeable seed dormancy. Bot. Re., 44: 365-385.

Salter, W.J and Smith, J.M. (1986). Peas - control of establishment pests and diseases using metalaxyl based seed coatings. Proceedings of the British Crop Protection Conference, November 17-20, 1093-1100.

Schloman. Jr. and Wanger, J.P. (1991). Rubber and coproduct utilisation. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 287-310.

Scott, J.M. and Blair, G.J. (1988). Effect of source and rate of phosphorus on emergence and early growth of phalaris (*Phalaris aquatica* L) and lucerne (*Medicago sativa* L). Aust. J. Agric. Res. 39(3): 437-445.

Scott, J.M. (1989). Seed coatings and treatments and their effects on plant establishment. Adv. Agron. 42: 43-83.

Shin, D.H., Yang, J., Chow, K.S. and Han, K.H. (1999). A *Hevea brasiliensis* homolog of DnaJ (HvDnaJ) is expressed abundantly in latex and induced by ethylene. Plant Physiol. 119:363.

Siddiqui, I.A. and Locktov, P. (1981). A feasibility study on the commercialization of guayule in N.S.W., Australia. Division of Plant Industry, California Department of Food and Agriculture, Sacramento, California.

Siddiqui, I.A., Connell, J.L. and Locktov, P. (1982). Report on the feasibility of commercial development of guayule in California. Division of Plant Industry, California Department of Food and Agriculture, Sacramento, California.

Siler, D.J. and Cornish, K., (1994). Hypoallergenicity of guayule rubber particle proteins compared to Hevea latex proteins. Ind crops prod., 2; 307-313.

Smith, A.E. and Miller, R. (1987). Seed pellets for improved seed distribution of small seeded forage crops. J. Seed Technol., 11: 42-51.

Smith, H. (2000). Phytochromes and light signal perception by plants-an emerging synthesis. Nature. 407; 585-591.

Stewart, G.A. and Lucas, S.M., (1986), Potential production of natural rubber from guayule (*Parthenium argentatum*) in Australia. CSIRO, Australia.

Stone, J.D. and Fries, J.N., (1986). Insect fauna of cultivated guayule, *Parthenium argentatum* Gray (Campanulatae: Compositae). J. Kans. Entomol Soc., 59; 49-58.

Sundar, D. and Reddy, A.R. (2001). Interactive influence of temperature and growth light intensity on rubber accumulation and rubber transferase activity in guayule (*Parthenium argentatum* Gray). J. Plant Physiol., 158; 1291-1297.

Taiz and Zeiger, (2002). Plant Physiology, Third Edition

Taylor, A.G., Klein, D.E. and Whitlow, T.H. (1988). Solid matrix priming of seeds. Scientia Hort. 37: 1-11.

Taylor, A.G. and Harman, G.E. (1990). Concepts and technologies of selected seed treatments. Annual Review of Phytopathology, 28: 321-339.

Taylor, A.G., Allen, P.S. Bennett, M.A., Bradford, K.J., Burris, J.S. and Misra, M.K. (1998). Seed enhancements. Seed Sci. Res. 8: 245-256.

Teetor, V.H., Ray, D.T. and Veatch, M.E. (2003). Concentration of resin, rubber, and guayulins in various parts of guayule plants. Association for the advancement of Industial Crops. 12-15 October 2003. Portland, Oregon USA.

Thompson, K. and Grime, J.P. (1983). A comparative study of germination responses to diurnally fluctuating temperatures. *J. Appl. Ecol.*, 20, 141-156.

Thompson, A.E. and Ray, D.T. (1989). Breeding Guayule. Plant Breed. Rev. 6: 93-165.

Tipton, J.L., Craver, J.L. and Blackwell, J. (1981). A method for harvesting, cleaning, and treating achenes of Guayule (*Parthenium argentatum* Gray). HortSci., 16(3): 296-298.

Traub, H.P. (1946). Concerning the function of rubber hydrocarbon (caoutchouc) in guayule plant, *Parthenium argentatum* Gray. Plant Physiol. 21:425-444.

Trewin, D. (2003). ABS Catalogue No. 1301.0 - 2003 Year Book Australia. Australian Bureau of Statistics, Canberra, Australia.

Vanden Born, W.H. (1971). Green foxtail: seed dormancy, germination and growth. *Can. J. Plant. Sci.*, 51, 53-59.

Varty, K., Arreguin, L.B., Gomez, T.M., Lopez, T.P. and Gomez, L.M.A. (1983). Effects of abscisic acid and ethylene on the gibberellic acid-induced synthesis of α-amylase by isolated wheat aleurone layers. Plant Physiology. 73: 692-697.

Wanger, J.P. and Schloman Jr. W.W. (1991). Processing. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 261-286.

Watkinson, J.I and Pill, W.G. (1998). Gibberellic acid and presowing chilling increase seed germination of Indiangrass (Sorghastrum nutans (L.) Nash.). HortScience. 33(5): 849-851.

West, J., Rodriguez, E. and Hashemi, A. (1991). Biochemical evolution and species relationships in the genus *Parthenium* (Asteraceae). In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 17-31.

Whitworth, J.W. (1981). Direct seeding of guayule. Proceedings of Second Annual Meeting of the Guayule Rubber Society: 5p.

Wood, M.A. (1997). Guayule Latex process is licensed. News, Agricultural Research Service, USDA. Available at: http://www.ars.usda.gov. Accessed on 31 Aug. 2003.

Wright, N.G., Fansler, S. and Lacewell, R.D. (1991). Guayule Economics. In: Whitworth, W.J and Whitehead, E.E. (Eds.). Guayule Natural Rubber. Office of Arid Land Studies, The University of Arizona, USA, pp. 351-365.

Yamaguchi, S. and Kamiya, Y. (2002). Gibberellins and light-simulated seed germination.

Ykema, R.E. and Stutz, J.C., (1991). Isolation, identification, and pathogenicity of *Fusarium* spp. from Guayule in Arizona. Plant Dis**.,** 75; 736-738.