EFFECT OF SMOKE SOLUTION ON PERFORMANCE OF *Pinus elliottii* AND *P. taeda* SEED.

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ABSTRACT

In recent years research has shown that exposing seed to smoke or smoke solutions results in increased germination of some, but not all the species tested. Tests showed that exposing seeds of some commercial crops to smoke increased early plant growth. The stimulatory effects of smoke were shown to benefit the germination of species regardless of whether or not fire played a part of the species ecological cycle.

In commercial forest nurseries any method of increasing the recovery rate of seed presents opportunities for realising savings of related production costs. Greater recovery rates of genetically improved seed present opportunities for capturing more related growth in field operations.

Improving efficiencies of seed recovery at an early point in the production chain have multiplied effects further on down the chain.

Two species of pine, namely *Pinus taeda* and *Pinus elliottii* that do not generally present high rates of germination were selected to test if applications of smoke solution could increase germination or emergence rates by more than 5%. As seed of both species are known to respond positively to existing seed pre-treatments the effects of smoke needed to be tested in combination, and apart from the pre-treatments. A secondary aim of the study was to examine the effect of smoke on early plant growth.

Attempts to optimizes the concentration of the smoke solution were not undertaken as part of this study, as a rinsing treatment, included in the trials, is known to remove any inhibitory effects of a high concentration of the smoke solution. Tests to determine the variability of the seedlots was carried out for statistical purposes. The interaction between smoke application and pre-treatments were tested, firstly in Petri dishes under controlled environmental conditions, and then in nursery trays under standard commercial nursery conditions for both species.

The inclusion of smoke in combination with the **target moisture stratification** (TMS and rinse pre treatment) had a significantly positive effect on *P. taeda* in a controlled environment. The same combination did not yield a positive results when tested under nursery conditions. Recommendations are made regarding future tests to see if the beneficial combination found in the controlled environment could be replicated under

nursery conditions. Further motivation for conducting the tests exists in that the particular combination set gave significantly better early plant growth under nursery conditions. No other combinations tested yielded positive results.

Declaration

I hereby declare that the research work reported in this dissertation is the result of my own investigation, except where acknowledged.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 The role of tree seed in the forest industry

Commercial forest industries continually seek to improve the efficiencies of their operations. One way of improving these efficiencies is to improve the yield of timber that can be harvested from a given area over a set time. Tree improvement programmes are one of the methods used to achieve this end. These programmes select trees that have the ability to yield timber more efficiently. The improved material is propagated for operational deployment in commercial nurseries through seed or vegetative propagation methods. Vegetative propagation is relatively a more complex and costly means of production. By the nature of the industry, a relatively small number of top performing individual genotypes are selected and then deployed as clones. Unless the clone has been thoroughly tested by environment interaction deployment by vegetative propagation may not yield any benefit to the grower. The disease and insect infestation risks are much greater when using clonal material. This is because the crop lacks the genetic diversity inherent in a crop raised from seed. Although there is a slow growth in the proportion of clonal material being deployed throughout the industry, the large majority of forest propagules that will be deployed in the foreseeable future will be raised from seed. This study, therefore, deals with aspects of using seed as a method of propagation.

The increased yields of timber, in terms of both quantity and quality, with comparative input costs, are a major driver of commercial forestry profitability. Improved seed can yield up to 44% more tonnes of Kraft pulp over unimproved seed sources (Morris 1994). Invariably, the demand for the newly developed and the most improved material exceeds the supply. Once the superior yielding material has been identified it has to be to be bulked up to establish seed orchards. Seed orchards only come into production slowly as the trees mature. In the first eight to twelve years the trees tend to only yield small quantities of seed

(Barnes and Mullin 1974). It is therefore understandable that any forestry enterprise would want to maximise the recovery from the scarce supply of improved seed.

In South Africa commercial timber plantations cover 1 339 282 ha. Of this, softwoods cover 54% of the area (Department of Water Affairs and Forestry 2004/5). Forest tree seedlings are commonly raised from seed and dispatched in seedling trays. The customers require that there be one seedling per cavity. The seedling producers can make the most efficient use of their seed if they can sow one seed per cavity. In order to adopt this practice it is necessary that a high percentage of the seed must have the ability to germinate. Every cavity in which a seed fails to germinate represents inefficiency in terms of nursery space, trays, growing media and other input utilisation. Any treatment that can increase the germination of improved seed will have the effect of increasing the nursery's efficiency and the forestry industry's profitability. The amount of pine seed required annually in South Africa and proportional distribution of plantations are shown in Table 1.1.

Table 1.1 Annual seed requirements for some pine species in South Africa. (Department of Water Affairs and Forestry 2004/5)

Species	Total area planted to each species in SA (ha)	% of total area planted to each species in SA (ha)	Total area needing replanting annually (ha)	The No. of seeds needed annually per specie, for re-establishment. See a, b, below	The No. of seeds per kg for each species	Estimated amount (kg) of seed needed annually for entire replanting programmed in SA
P. patula	351674	53.3	14788	36969569	100000	369.70
P. elliottii	183571	27.89	7719	19297818	25000	771.91
P. taeda	42122	6.4	1771	4428056	45000	98.40

a. Assuming all areas are re-planted with the same species.
b. Based on 2500 seedlings per ha.

1.2 Nurseries and seed usage

Sappi Forest Research Nursery Manual (2004) sets 75% recovery as being an expected target for seed usage. Over a five year period (2001 – 2005) Sappi's recovery for the following species was:
P. patula = 55%, *P. ellottii* = 56% and *P. taeda* = 33. These recovery percentages are lower than the 75% figure used to calculate the total South African annual seed usage.

Other commercial forestry companies, i.e. Mondi Forests and Komatiland Forest North and Global Forests, raise some of their own seedlings but also buy in a substantial proportion of seedlings required from private nurseries.

1.3 Status of pine seed research in the South African forest industry

There is very little research that has been done on pine seed in the industry in South Africa in the last three decades. Some research was done on pine seed at the University of Stellenbosch by Dr. D G M Donald before 1977. Sappi Forests have done some research on how to obtain acceptable recovery from seed. This information is in the form of in-house trial reports and is not in the public domain. Some aspects of the research are detailed below. The knowledge pool available is from various publications or information gained from forestry organisations who deal with pine seed in the northern hemisphere countries. The Institute of Commercial Forestry Research have not focused on pine seed research.

1.4 The focus on the efficient use of pine seed

Sappi has conducted some multi-disciplinary investigations into seed use efficiencies within its nursery operations. The findings and recommendations from these investigations have been recorded in internal company reports. If the recommendations contained in the reports are followed, nurserymen should be able to make efficient use of their seed.

In processing seed into seedlings, four basic processes are involved:

a) The seed pre-treatment process: Any treatment given to the seed prior to sowing that is intended to enhance its nursery performance.

b) The seed sowing treatment: Methods and conditions the seed will be exposed to in-planting the seed.

c) Seed emergence management: This includes the general and special treatment given to the seed after sowing to encourage the seed to germinate and emerge.

d) Seedling culture management: This would include all the practices observed in raising a newly emerged seedling to the point it is considered saleable as a seedling (i.e. watering, fertilising, disease control, etc.).

The total efficiency is a factor of the efficiencies in each process. However, a poor efficiency in the first process has the greatest effect. This is because its effect is compounded down through the subsequent processes. Thus it is desirable to eliminate or reduce efficiencies in the earlier stages as this will have the greatest compound effect.

1.5 Existing work on seed pre-treatment

Seed pre-treatment for *P. patula, P. elliottii* and *P. taeda* has received considerable attention in the past. All three species have been grown on a large commercial scale in South Africa for many years. *P. elliottii* and *P. taeda* are widely grown in the south eastern United States and elsewhere in the world.

Containerised nurseries have become widely used in South Africa since the mid-1970s. This created a new awareness of the importance of obtaining good germination.

Subsequently the various known seed pre-treatments were reinvestigated by Sappi Research staff.

Aspects of seed pre-treatment investigated have been:

a) Scarification: Any treatment that may alter the seed coat in order to enhance germination and or emergence.

b) Stratification: Any cold storage or cold storage in the presence of moisture that may enhance germination or emergence.

c) Priming: Moistening the seed with water or with water and an additive, then re-drying the seed prior to sowing.

d) Imbibing the seed: The process of regulating how and when the seed takes up moisture in the initial stages of germination.

e) Light regimes: Regulating the amount of daylight and dark hours the seed is exposed to during germination.

From these investigations Sappi has produced a nursery manual tabling the best known nursery procedures, to aid their nurserymen in running their nurseries efficiently (Sappi Forests Research Nursery Manual, 2004).

Within Sappi, aspects of raising seedlings, other than seed pre-treatment, which have received attention include seed sowing techniques, effects of fungicides on seed germination, effects of different composted bark sources, seed moisture content and storage, hand versus machine sowing and effects of bark medium sterilisation. Unlike the seed pre-treatments, there is not much information in the public domain. The processes are largely managed at the discretion of the nursery managers. Some undocumented

information has been made available to nurserymen through the sharing of information within special interest groups such as the Seedling Growers Association of South Africa.

1.6 Areas of opportunity to improve efficiency with regard to seed recovery

Pine seed giving over 80% germination is considered to be an excellent seed recovery by forest trees standards (Hammon 2002).

Data from routine germination trials carried out under controlled conditions at Sappi's Shaw Research Centre (SRC) produced the data shown in Table 1.2. This information was generated under controlled laboratory conditions. The nursery emergence results are considerably lower (*P. patula* 55%, *P. elliottii* 56% and *P. taeda* 33%). See Section 1.2.

Table 1.2 Germination data for the major pine species in South Africa

Species	Control (%)	Imbibed (%)	Pre-germinated (%)	Target Moisture-Stratified (TMS) (%)
Pinus patula	85.6	86.2	91.2	*
Pinus elliottii	55.2	76.8	79.82	68
Pinus taeda	59.2	77.8	79.4	82.5

* TMS testing was not carried out on *P. patula.*

As stated earlier, the total efficiency is a factor of the efficiencies in each process. The magnitude of the effect any inefficacy has on the total process will depend on where in the chain of events it occurs. The higher up in the chain of events the more significant is its effect. This is because the detrimental effect is compounded on down through the rest of the processes. From the above germination results it can be surmised that there is inefficiency at the beginning of the nursery process in that in *P. ellottii* and *P. taeda* between 20.2% and 17.5 % of the seed does not germinate in ideal laboratory conditions and these seem to be considerably worse in the nursery environment.

1.7 Justification and objectives

In recent years, research has been conducted on the use of smoke and smoke solutions in order to increase germination percentage of many species. (Brown 1993a; Baxter and Van Staden 1994; de Lange and Boucher 1990).

If by applying smoke as an additional or alternative seed pre-treatment to our pine seed, a greater overall amount of germination is obtained, this may be economically significant for the following reasons:

a) It will reduce the amount of seed used by the nurseries.
b) It will improve overall nursery efficiency.
c) It will allow better usage of the scarce improved material which is critical in improving the overall efficiencies of the forest industry.

From the above average seed germination results (Table 1.2) it can be seen there is room for potential improvement. Therefore, the objective of this study was to determine whether the application of smoke solutions, in combination with various standard and non-standard methods of raising seedlings, would significantly increase the germination and growth of two commercial pine species, namely *P. elliottii* and *P. taeda.*

The trials aimed to examine the effects and interactions of adding a smoke solution to existing standard seed pre-treatment methods and to test the effects that smoke solution have on seed germination under both controlled environmental and nursery conditions. In testing the seed under nursery conditions, two different methods of applying smoke solution to the bark media, in combination with the seed pre-treatment methods, were tested. Treating the bark media with smoke solution aimed to determine the best method of adding smoke solution to seed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

De Lange and Boucher (1990) were the first to publish their findings that smoke and extracts of smoke stimulated seed germination of a fynbos species, *Audouinia capitata*. Following this significant publication, Brown (1993a; 1993b) showed that other species from other genera and families also exhibited improved germination with smoke treatments (Light and Van Staden 2004).

The effect of smoke on agricultural seed quality was discovered by subsistence farmers long before the recent upsurge in scientific interest in this field (Modi 2002). It was known that seed stored over fire and smoke showed higher germination and vigour than seed stored elsewhere (Modi 2004).

Initially studies on smoke-stimulated seed germination focused on plants that have fire as a part of their ecological cycle. Studies have shown that smoke is effective on species from a wide range of families, varying in their ecology, reproductive methods, seed size and morphology (Brown 1993a; Dixon and Roche 1995; Pierce et al. 1995; Roche et al. 1997a; Van Staden et al. 2000).

2.2 Effects of smoke and seed germination from an ecological point of view

According to Brown (1993a) propagation of fynbos from seed is difficult, as seed of many species are dormant at harvest maturity, and require very specific environmental cues for germination. Fynbos occurs in a mediterranean climate and is subjected to the stresses of summer drought, low fertility and periodic fires. The fire frequency can vary between one and forty years and is a naturally occurring phenomenon in fynbos. Fires provide the major

cues for germination and it is ideal to reproduce these conditions when trying to germinate wild flower seed.

The heat from fires can stimulate germination, especially of those seeds with hard impervious seed coats. However, for a wide range of species, smoke can independently stimulate seed germination (Brown & Botha 2004).

Out of 220 species tested, 54% showed a significant improvement in germination following smoke application (Brown 1993a; Brown and Botha 2002). Studies on Californian and Australian species occurring in mediterranean climates showed the response to be widespread (Dixon and Roche 1995; Dixon et al. 1995; Keeley and Bond 1997; Tieu et al. 1999). The increase in germination percentage when seeds are exposed to fire and smoke can be regarded as a survival mechanism. The seeds are likely to be released into environments suitable for subsequent seedling growth. These conditions would include areas exposed to high light conditions and areas that do not have a pre-existing plant cover (Lieshman and Westoby 1994; Westoby et al. 2002).

It is generally accepted that there is a relationship between seed size and successional status. Small- seeded species typically require open high light environments for establishment (Salisbury 1942; Swaine and Witmore 1988). Small-seeded species have limited reserves to persist in low light conditions (Lieshman and Westoby 1994, Westoby et al. 2002). Brown et al. (1993) postulated that there may be a relationship between seed size and response to smoke. Small-seeded species may be most likely to respond to smoke since they may be most dependent on finding sites that are burnt, and thus free of competing vegetation. Smoke would indicate the potential presence of burnt sites (Brown et al. 2003).

During a fire event, seed would not only be exposed to smoke but possibly dry heat, wet heat, charred wood and smoke. Enright and Kintrup (2001) looked at the effect of these factors on the germination of dormant soil-stored seeds from *Eucalyptus* woodland in

Western Australia. Fifty-nine species were included in the trial. All treatments with the exception of charred wood, gave a significant increase in seed germination compared to the control. The smoke treatment gave the highest number of germinated seeds. The species richness differed significantly among treatments but the highest mean richness (i.e. the greatest number of different species that emerged) was recorded for the smoke treatment. The control and charred wood treatment gave the lowest mean richness. Heat was a specific requirement for triggering germination in hard-seeded species. Smoke proved the most effective trigger for seed from a broad range of other families. The experiment raised the issue of the potential of confounding effects of the physical and chemical processes when smoke was used as a germination trigger (Enright and Kintrup 2001). Read et al. (2000) found that the speeding up of the germination was the major response in some species and this may represent an adaptive advantage for short-lived ephemeral species where they could germinate and grow in a 'low competition' window of time. In the study of Enright and Kintrup (2001) no similar trend was found. Speed and uniformity of germination in response to smoke holds potential benefit for commercial seedling growers.

Brown et al. (1994) conducted a study in which seeds of 32 species of fynbos were screened to determine how important the smoke cue is for germination. Twenty-five of the 32 species tested showed a statically significant improvement. Untreated seed of 18 of the species showed low germination percentages (between 0.1 and 0.2%). These results showed that, under natural conditions, smoke is an important cue for triggering germination in these species. Four of the species that did not germinate well had hard seed covers (nut) and possibly require heat or different additional germination cues. In collating information on 67 species of Cape Restionaceae for which data was available, 42 (63%) gave a significant germination response to smoke treatment (Brown et al. 2003). Of these species 15 gave a response of 100% or greater. This suggested that smoke is the overriding cue for seed germination. Thirty species gave a response of less than 100% and Brown and Botha (2004) suggest that smoke is possibly one of a number of germination cues that are important.

Close and Wilson (2002) investigated provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensis* seed. Both these eucalyptus species are important commercial species for quality wood pulp in south-eastern Australia. Close and Wilson (2002) reported that when seed (prior to harvest) is aerially sown in over-logged and burnt coupes the stand re-establishment is often less than 80% of the target density. Poor germination and establishment of the seedlings is regarded as a major cause of the sub-optimal stand density. The stands can be re-established from plants raised in small pots or plugs. Nurseries anticipating low germination percentages often sow 3-4 seeds per plug. After germination the plugs require manual thinning or filling in order to get one seedling per plug. Seedlings thinned out are transplanted into empty plugs by pricking the seedlings in. The pricking process often results in seedlings with root deformities, which are associated with poor growth and survival after transplanting.

Some species of eucalyptus such as *E. regnans* and *E. delegatensis* have high levels of seed dormancy. Pryor (1954), and Close and Wilson (2001) investigated possible causes and methods to alleviate seed dormancy in *E. regnans* and *E. delegatensis*. One of the issues addressed was whether exposing the seed to smoke would be an effective germination pre-treatment. *E. regnans* occurs naturally in low to medium altitudes (100-900 m) and *E. delegatensis* occurs at higher altitudes (500-1200 m) in Tasmania (Boland et al. 1980). Close and Wilson (2001) reported that smoke generated by burning dried leaf material, collected from the seeds origin in a standard bee keeper's smoker, was pumped through a water-cooled aluminium pipe. The cooled smoke flowed into a plastic tent housing the imbibed seed. The seed was supported on wire racks. A chimney in the tent ensured that a constant flow of smoke passed the seed. After smoking the seed for 45 minutes the seed was transferred to a germination chamber. The results showed that no differences were detected between the germination of the smoked or non-smoked seed. Close and Wilson (2002) concluded that unlike many post-fire occurring species (see Dixon et al. 1995) dormancy in these species is not overcome by exposure to smoke. Close and Wilson (2002) postulated that the rapid recruitment of *E. regnans* and *E. delegatensis* seedlings after a fire was mainly due to accelerated seed dropping into the fertile ash bed.

The ash bed would be conducive to successful seed germination and seedling establishment (Cremer 1965).

Enright and Kintrup (2001) conducted experiments on the effects of smoke, heat and charred wood on the germination of dormant soil stored seed from a *Eucalyptus baxteri* heathy woodland in Victoria State, Australia. The effects of wet heat, dry heat, smoke and charred wood on the germination of soil stored dormant seed was tested. Charred wood was included as a treatment as it was noted that charcoal and aqueous extracts of charred wood were reported to act as a cue for a variety of fire annual and partially opportunistic plants across a range of families native to the Californian chaparral (Keeley and Nitzberg 1984).

Smoke was included as Keeley and Fotheringham (1998) had reported that the germination of several chaparral species was enhanced by the action of nitrogen compounds in smoke. The study of Enright and Kintrup (2001) describes and evaluates the impact smoke heat and charred wood had on the resulting species' richness, seedling density and species composition. The information from the experiment could prove to be useful as a management tool in managing fire prone environments. A better understanding of seed germination ecology may be valuable in conserving endangered plant species (Read et al. 2000).

One of the results of the study of Enright and Kintrup (2001) showed that smoke may be a more effective treatment than heat in enhancing the germination of dormant soil stored seeds. However, there was an overlap in the species triggered as the two factors do not stimulate germination across the same set of plant species. It was concluded that heat was a specific trigger for some species, especially hard-coated seeds, whereas smoke appeared to trigger others. A third group (the largest) responded to heat and smoke. It was noted that there existed interpretational problems why the same species could respond to two different triggering mechanisms, namely, smoke (a chemical trigger) and heat (a physical trigger). Similar findings were reported by Enright et al. (1997) and Read et al. (2000) that heat treatments are likely to stimulate chemical triggers as constituents from the soil organic

matter would be released during heating. Analysis of the water from the heated soil contained a number of the same compounds that were present in smoke derived from burning above ground vegetation (Morrison and Morris 2000). This finding supports the idea that it may be better to test for the effects of smoke in artificial settings that allow for the separation of effects of heat, and smoke. The question, amongst others, remained unanswered, as to what fraction of the ecosystem's organic matter is primarily responsible for the smoke germination response (Morrison and Morris 2000).

2.3 The effect of smoke from a seed physiology point of view

It is clear from the preceding discussion that smoke influences germination of a number of species. Prior to July 2004 the identity active compound(s) was unknown. A number of studies have reported on the ongoing search for clues as to the physiology of the induction of germination by plant derived smoke (Brown and Van Staden 1997; Van Staden et al. 2000). The principal(s) active in smoke seem to have the ability to stimulate seed germination (de Lange and Boucher 1990), somatic embryogenesis (Senaratna et al. 1999), flowering (Keeley and Fotheringham 1998) and rooting (Taylor and Van Staden 1996). Smoke solutions appear to behave in a manner similar to plant growth regulators (Senaratna et al. 1999; Gardner et al. 2001).

Thomas and Van Staden (1995) suggested that the active compound of smoke may act by modulating sensitivity to endogenous growth regulators via the modification of receptor molecules or activation of receptor molecules. Some researchers, in contrast, have suggested that the promotion of seed germination by smoke is the ability of smoke solutions to alter the integrity of the endosperm membrane or the seed coat Keeley and Fotheringham (1998). There may also be an interaction of smoke and phytohormones (Van Staden et al. 1995c). Van Staden et al. (1995c) suggested that smoke may act synergistically with gibberellins (GAs). GAs mitigate the suppression of germination caused by the fruit wall. GAs also induce structural modifications to the endosperm of the seed prior to the emergence of the radicle (Psaras and Georghiou 1983).

Part of the breaking of dormancy in photoblastic seeds is under phytochrome control (Bewley and Black 1982; Thomas 1992). Gardner et al. (2001) conducted a study in an attempt to gain understanding of the interaction between smoke, red light and endogenous gibberellins. This was done by examining the nature and the sequence of the changes the various cues solicited and their dependence on various conditions. Gardner et al. (2001) commented that the key to understanding the process underlying the process of induction of germination lay in the characterisation of the relationship between temporal patterns of physiological and developmental changes. The study by Gardner et al. (2001) aimed to determine if smoke substituted for far-red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism. The study found that an anomaly in the timing of the response lettuce seed had to red light and smoke could indicate that the two cues had different physiological modes of action. It was suggested that the nature of the difference in the response seed treated with smoke and red light had in comparison to seed treated with far-red light could indicate that smoke acts via the phytochrome system. The results of the study by Gardner et al. (2001) show a clear indication that smoke-promoted germination can be prevented by far-red light treatment. It is possible that the active component(s) of smoke may act as a powerful plant growth regulator (Bewley 1997).

A study in order to gain more insight into the nature of smoke-stimulated seed germination was carried out by Light et al. (2002) and reported a dual regulation of germination of lettuce seed by smoke solutions and exposure to light regimes (Light et al. 2002). The results provide a clear indication that smoke-promoted germination can be effectively prevented by far-red light treatment and at least part of the mechanism of smoke action is phytochrome-dependant. The treatment of Grand Rapids lettuce seeds with aqueous smoke solutions of differing dilutions produced a response curve similar to that seen with phytohormone response curves (Drewes et al. 1995).

At high concentrations (dilutions of 1:100 and higher) the smoke extracts may become inhibitory to germination. At dilutions lower than 1:100 the treatment significantly

increased germination, in comparison to the water controls. Baldwin et al. (1994) found that the stimulatory effect of smoke was irreversible. The germination cue could not be removed by rinsing the seeds in water. However, the inhibitory effect of high smoke concentrations appears to be reversible (Brown. 1993a). Brown (1993b) showed that seeds of *Syncarpha vestita* treated with a 1:2 concentration of smoke extract did not germinate. Flushing this seed with distilled water, however, relieved the inhibitory effect of the high concentration. Brown (1993b).

Light et al. (2002) showed that the duration of the minimum exposure time to smoke solutions in order to obtain the best germination was determined. Storage treatments were also included to determine if there was a detrimental effect of storing seed exposed to high concentrations of smoke extract for long durations. Testing the storage effects of seed that were pulse-treated to see if the seed could retain the germination cue was also tested. Certain pulse-treatments showed significantly higher germination than seeds exposed to smoke solutions for 24 h. The study showed that for certain treatments, seed stored for two weeks showed a similar germination response to seeds which were allowed to germinate immediately after an equivalent treatment. Further experiments showed that when seeds had been given certain applications of smoke extract, rinsed and dried, those stored for two weeks gave an improved germination over those sown immediately (Light et al. 2002). Other significant findings of the study were that the promotion of germination by smoke solutions is dependent on the initial period of rapid imbibition. It is likely that prior imbibition of seed in water reduces the ability of the smoke solution to reach certain cells in the embryonic axis. These cells are thought to play a central role in the induction of germination. The reduction of germination by pre-treatment with water indicates that either the active compound from smoke is needed at a specific threshold level, or the water somehow created a barrier to solute movement (Light et al. 2002). Seeds that were imbibed for periods longer than 2 h, had a higher rate of germination response than those imbibed for 1h. This could be attributed to the removal of germination inhibitors of seed that has imbibed for long enough, or the seed is rinsed after imbibition. The pulse treatments showed that there needs to be a minimum period of exposure to the smoke cue in order to

obtain a threshold level of the active component(s) in the embryonic axis (Light et al. 2002).

That exposure of seed to increased concentrations for reduced times had a good effect, confirms the above finding. When seeds were pulse-treated for periods exceeding 2 h, the roughly linear increase in germination percentage supports the idea that the active principal in the smoke solution activates the germination signaling mechanism. Light et al. (2002) argued that this result supports the idea that the smoke solution does not work by purely physically changing the seed membrane structure and suggests that other mechanisms are involved in smoke-induced germination of lettuce seeds. The prolonged exposure to smoke solution resulted in a gradual inhibition of germination. This could point to the presence of small amounts of toxins or other compounds (which if exposed to for long enough) could suppress germination (Light et al. 2002).

Previous investigations have shown that exposing the seed to high concentrations of smoke extract for prolonged periods inhibits germination (Drewes et al. 1995). These findings indicate that the best results are obtained when seed is given a short pulse treatment of a solution of high concentration of smoke extract. When these seeds were well rinsed the detrimental effects were no longer apparent. The rinsing apparently removed the inhibitory compounds of the smoke. A 1:10 solution irreversibly damaged the seed by rupturing the seed coat and expanding the cotyledons (Light et al. 2002).

The removal of the inhibitory effects after rinsing shows dual regulatory cues in smoke. Light et al. (2002) concluded that "this competitive interaction, in which the germination promoter cannot be leached while the inhibitor may be important in post-fire environments - providing a mechanism to prevent germination until sufficient rainfall leaches the inhibitory compounds and then allowing the stimulatory compound(s) which are active over a broad concentration range, to function."

Nitric oxide (NO) and related nitrogen oxides have been reported as stimulators of seed germination in a number of studies and it has been suggested that these compounds are responsible for the promotion of germination by smoke (Light and Van Staden 2003). Thanos and Rundel (1995) reported that nitrates stimulated the germination of two post-fire annuals, *Emmenanthe penduliflora* and *Phacelia grandiflora*. The study concluded that the principal factor for inducing germination was nitrate. Keeley and Fotheringham (1997a), in another study, showed that nitrogen oxides induced 100% seed germination of *E. penduliflora* seeds in a manner similar to smoke. NO_2 had a greater stimulatory effect than NOx ($NO+NO_2$) and induced germination directly and indirectly. The large amounts of nitrogen oxides generated by wild fire's combustion of organic matter triggered the germination of *E. penduliflora* (Keeley and Nitzberg 1984).

In another study conducted by Keeley and Fotheringham (1998), the induction of smoke induced seed germination in post-fire Californian chaparral did not appear to be triggered by the presence of increased nitrates. The existing nitrate concentrations in the biomas smoke were effective in inducing germination. Recently there has been much research on the role of NO in plant cells and NO is considered an important signal and "effector molecule" during the development in adaptive plant responses (Light and Van Staden 2003). NO promotes seed germination and de-etiolation, and inhibits hypocotyl and internode elongation. These responses are light inducible in plants (Beligni and Lamattina 2000).

The study by Thanos and Rundel (1995) found that traces of NO found in smoke and smoke solutions possibly do play a role in signaling germination. This is seen in the number of species that respond to treatments of NO-releasing and other nitrogenous compounds. Light and Van Staden (2003) postulated that NO is probably not the only factor present in smoke which could be responsible for stimulating germination and concludes that "factors, other than NO are responsible for the enhanced germination of Grand Rapids lettuce seeds by aqueous smoke solutions" (Light and Van Staden 2003).

2.4 Smoke as a powerful wide-spread germination cue

Minorsky (2002) reviewed the literature available on the role of smoke as a widespread germination cue and from the review the following points have been summarised:

a) At the time of writing it was apparent that the exact manner in which the compounds contained in smoke work is not totally understood. What was known is that the combustion of dry or green plant material from many sources produce compounds that stimulate germination of seeds from many species.

b) The compounds that effect germination are apparently produced at around 160-200°C.

c) The remarkable effects that smoke solutions have on seed germination have found wide application, with more species responding positively than not.

d) The positive effect of smoke on seed germination is not limited to species native to fire-prone habitats (Pierce et al. 1995). In many species the effect of smoke is astounding. Smoke has been reported to enhance germination of *Erica cavisepala* and *Restio festuciformis* by more than 7000% and 25000%, respectively.

e) There exists a complex species interaction between smoke and other environmental factors.

f) In certain cases, smoke is a better enhancer than heat, while in others smoke and heat work synergistically to promote germination. Other factors such as seed age, light levels, temperature and degree of hydration influence the effect of the smoke induced germination.

g) The ability of smoke to enable seeds to rapidly overcome dormancy is long-lasting. The enhanced ability of seed treated with smoke extracts has been shown to be effective even after one year of storage.

2.5 The site of action of smoke

Seed dormancy is one of the most extensively researched areas in plant biology with well over 700 publications in the last ten years (Bewley 1997). Even with this amount of information many of the reasons why radicle protrusion is blocked, and thus the seed remaining in a dormant state, is not understood.

Available evidence indicated that dormancy can be located in one of two primary locations. These can be either in the embryo covering structures or within the embryo. Mechanisms within the covering structure may involve mechanical, permeability and chemical barriers to germination. Mechanisms within the embryo may involve the expression of certain genes, levels of certain plant growth regulators, the mobilisation and utilisation of food reserves and the activity of certain respiratory pathways. Some embryos may be too immature to germinate immediately and must undergo a further growth phase before germination is possible. It is possible that an individual species could have one or more of these dormancy mechanisms (Adkins et al. 2001).

In that some species may have one or more dormancy mechanism some species may respond to one or more or a combination of germination cues. Enright and Kintrup (2001) found that while smoke was the most effective trigger for a broad range of species from a broad range of families, physical triggers, (e.g. heat) could trigger germination in the same species. Heat was a specific requirement for triggering germination in hard-seeded species. Studies of the anatomy of smoke-stimulated seed may differ from most heat-shock-stimulated seeds in that most have a highly textured outer coat, a poorly developed outer cuticle, the absence of a dense palisade tissue and a semi-permeable sub-dermal membrane (Enright and Kintrup 2001).

It is known that both scarification and stratification may help reduce dormancy in pine species. Whether smoke or a combination of smoke and scarification or stratification will reduce dormancy effects is unknown. In a study published by Gardner et al. (2001) the peak levels of GAs in smoke- treated seeds were substantially higher than in red-light-treated seeds, which shows that GA synthesis is likely to be involved in the mechanisms underlying smoke-induced germination.

2.6 The search for the active component in smoke

Aqueous solutions of plant-derived smoke contain a complex mixture of thousands of components (Adriansz et al. 2000), thus it is not surprising that early studies have yielded divergent findings as well as several candidates. It has been questioned if burning or heating of different plant materials generates the same active compound(s). Van Staden et al. (1995a) concluded that similar types of compounds are present in smoke extracts derived from different materials. Seven out of twelve active compounds were found in both solutions formed from burning *Themeda triandra* and *Passerina vulgaris* (Van Staden et al. 1995b). The finding that the burning of cellulose alone has a stimulatory effect raised the possibility that the thermal breakdown of cellulose or hemicellulose may release one of the bio-active components (Preston and Baldwin 1999). What is known is that the cues are stable, water-soluble and active at low concentrations.

Flematti et al. (2004) reported that recently many attempts have been made to determine the compound(s) from smoke responsible for triggering seed germination. He suggested some of the key data that characterise a germination-promoting compound found in cellulose-derived smoke. Flematti et al. (2004) published a paper on the identity of a germinating compound present in plant and cellulose-derived smoke. This compound promoted germination of a number of plant species to a level similar to that observed with plant derived smoke water. The structure of this compound was shown to be that of a butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one).

The activity of the butenolide was demonstrated at very low concentrations (1 ppb) when tested on known smoke-responsive South African, North American and Australian species. The butenolide conformed to the necessary attributes of smoke that is produced from natural fires in natural environments. It is stable at high temperatures with a melting point of 118-119ºC. It is water soluble and showed capability to stimulate germination at a wide range of concentrations (1-100 ppt). The butenolide was derived from combustion of cellulose which is a component of all plants and universally present in all natural fires. It was further noted that (+)-strigol, which had been shown to promote germination of a parasitic weed (*Striga* species) is active at similar concentrations and contains a butenolide (Flematti et al. 2004).

Light et al. (2005) reported on the formation of a seed germination promoter synthesized from carbohydrates and amino acids. It was reported that heating proteins or amino acids with sugars at 180°C for 30 mins. produced water soluble extracts that promoted germination. Using high performance liquid chromatography it was found that the active fraction co-eluted with the active fraction from a smoke solution. Mass spectroscopy and gas spectroscopy showed that the active constituent is identical to the germination cue from plant-derived smoke. i.e. the same butenolide compound identified by Flematti et al.(2004) and also isolated by Van Staden et al (2004). These results showed that using a higher proportion of glucose resulted in extracts which gave higher levels of germination.

2.7 The potential of smoke in seed technology

Light et al. (2004) reviewed the potential of smoke in seed technology. Although the role of smoke as a germination cue has been previously reviewed (Brown and Van Staden 1997; Van Staden et al. 2000; Minorsky 2002) the review highlighted recent findings and focused on the application and potential of smoke technology in horticulture and agriculture. The review indicated that many indigenous species that are hard to germinate, especially fynbos species, can now be successfully propagated by seed. Notable examples are found in the Restionaceae species. Previously, Restionaceae species which were previously known to be

particularly difficult to germinate, although they have great potential in the landscaping and wild flower trade. *Syncarpha vestita* (Cape everlasting) can now be grown in cultivation and this hopefully will reduce the pressure on wild populations as a result of collecting (Brown 1993b).

The ability of seed to retain the smoke induced germination cue in storage is seen as a having important possible applications. Both Kirstenbosch Botanic Gardens (South Africa) and King's Park Botanic Gardens (Australia) have developed commercial preparations of smoke extract for treating seeds. The Kirstenbosch product is a filter paper which has been impregnated with a solution of smoke extract then dried. Seeds placed on the filter paper absorb the extract when water is added. The Australian product is a concentrated solution which can be diluted and used for treating seeds.

In agriculture, increased germination of lettuce and celery seed has been noted after using extracts of smoke. An aspect which has not fully been explored is the potential increased rate and synchronisation of germination of smoke-treated seed. This area holds a lot of potential for growers. Modi (2002) showed that smoke-treated maize seed had both a higher germination rate and a higher final germination than untreated seed. Smoke-treated seed produced more vigorous seedlings (heavier and taller) than untreated seeds.

It may be possible to use smoke as a tool in weed control by applying a smoke extract in order to stimulate a high proportion of the weed seed bank to germinate and subsequently killing the weeds prior to planting the desired crop. Smoke extracts have been used in habitat re-vegetation. Smoke extracts applied to mined areas in Western Australia resulted in 48-fold increase in the total number of germinates and a 4-fold increase of species richness. Successful re-vegetation using smoke extract would be related to the dynamics inherent in the soil seed bank (Read et al. 2000).

2.8 Post-germination effects of smoke-derived compounds

Sparg et al. (2005) indicated that the effects of smoke extend beyond germination, and can enhance seedling vigour. Blank and Young (1998) have also shown that smoke improves emergence and seedling growth in different grasses. The identification of the active compound allows for further research into the application of smoke technology on a variety of agricultural crops including maize. In a semi-arid region where stand density is a major constraint for maize crop production, this application is of particular importance (Murungu et al. 2003, 2005; Finch-Savage et al. 2004).

Van Staden et al. (2006) carried out a study to investigate the effects of smoke water and 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one on the germination and seedling development of tomato (*Lycopersicon esculentum* Mill. cv Heinz-130), okra (*Abelmoschus esculentus* (L) Moench. cv Clemson spineless), bean (Phaseolus vulgaris L. cv Dwalf Imbali) and a commercial maize variety (*Zea mays* L. var. Pan 6479. The study was extended to examine the use of this compound as a possible seed priming or pre-conditioning agent for maize. Results showed that smoke-water and butenolide significantly improved seedling growth, and increased both the root and shoot lengths of all the seeds in comparison to the control. The root lengths of tomato seedlings treated with butenolide were 10 times longer than roots of the control seedlings grown in water. Smoke-water and butenolide significantly increased root lengths in both okra and bean (up to three-fold, on average). In tomato and okra the difference in shoot lengths, between smoke-treated and butenolide-treated seedlings, was highly significant in comparison to the control. This suggests the smoke compound may stimulate cell elongation and/or division. With smoke-water and butenolide treated seeds, no significant improvement in rooting was observed in tomato, okra and bean when grown in a 16:8 hour light/dark cycle. This suggests butenolide may act as a root growth stimulant although the effectiveness may be affected by light in a similar manner to auxin. Both the butenolide and smoke water treated seeds had significantly higher fresh masses and showed greater vigour indices compared to untreated seed. Seedling mass of tomato and maize was significantly higher than untreated seedlings. These results suggest that the smoke-derived

compound not only stimulates germination but also promotes growth after germination. Eight-day-old smoke-water and butenolide–treated maize seedlings developed more roots in comparison to the control seedlings. Van Staden et al (2006)

Other findings reported, including one made by Brown (1993a), in which young seedlings of *Erica* species showed more vigorous vegetative growth after smoke-water treatment. These confirm that smoke treatment can result in improved seedling vigour. As a result of these findings, Van Staden et al. (2006) investigated the post-germination effects of smoke/butenolide treated maize and reported that both smoke- and butenolide-soaked seeds had a positive short term growth response. Other kernels were planted into pots where the media had been drenched with smoke-water prior to the seed being sown. Plants from these pots did not show a significant improvement on fresh root mass, but did show a significant greater mass of dry root and shoots, in comparison to the control. The drenching treatment significantly improved the shoot height of maize plants. This demonstrated that smoke solution and butenolide have promotory effects beyond germination.

The ability of seed to retain the smoke cue even after rinsing suggests that smoke can be utilised as an effective seed pre-treatment (Baxter and Van Staden. 1994). Pre-soaking can be long lasting and, in some cases, dry-stored seed sown after a year had lost none of the effect of the treatment (Brown et al. 1993). The rapid germination and emergence can help maize seedlings to escape from a number of environmental stresses (Murungu et al. 2005). Soaking maize kernels overnight, surface drying, and sowing on the same day is a practice that has been recommended by researchers (Harris 1996; Murungu et al. 2003, 2005). On the contrary, priming maize kernels may be detrimental due to rapid and prolonged imbibition, temperature fluctuations, and other environmental factors encountered under field conditions (Cal and Obendorf. 1972; Harrison 1973; Martin et al. 1991; Finch-Savage et al. 2004). Van Staden et al.(2006) found that soaking maize kernels for one hour in a smoke solution exhibited the stimulatory effects at germination, and post-germination levels. This indicated that smoke treatment of maize seed can minimise the soaking time

required for faster germination, and other stimulatory effects, without any of the detrimental effects caused by prolonged soaking.

Van Staden et al. (2006) concluded that the use of smoke compounds to pre-treat crop seeds is a potential breakthrough in the field of agriculture. It was also suggested that the newly-isolated compound from smoke be potentially used as a new growth regulator in various disciplines of plant science. From the literature reviewed there is sufficient evidence to indicate that applying smoke to pine seed may increase the overall germination of the two pine species under investigation, and may have a beneficial stimulatory effect on the post-emergence growth of the resultant seedlings.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Species used and source of seed

Commercial seed lots of *P. elliotti* (S/N 63676) and *P. taeda* (S/N 63822) were selected because they are an important species used by Sappi and other forest companies in South Africa. The seed for both species was obtained from commercial collections originating from a number of different parent plants. The seed from each species had been thoroughly mixed in order to ensure uniformity in the batch. The seed was stored in moisture proof plastic/cellophane laminated packets under refrigerated conditions. From previous experience of storing seeds of these species, it is reasonable to assume that the viability of the seed would have remained stable over the twelve month time period in which tests were conducted. These two seed lots could be regarded as typical of the commercial material available to the forest nursery industry in South Africa at the time of this study. The seed lots had been subjected to routine moisture and germination testing, with *P. eliottii* (SN 63637) having a germination percentage of 75.2% (dry sown) and moisture content of 9.85%, and *P. taeda* having a germination percentage of 66.4% and a moisture content of 2.23%. The moisture content of the *P. taeda* seeds might have been lower than desired, but it was the only seed lot available at the time.

3.1.2 Standard seed handling protocols

The methods used to test the seed lots were in accordance with the standard seed testing practices as laid out in the 2004 Sappi Forests Research Nursery Manual. The manual lays out the best operating practice to be used for each procedure. In the present study, the

procedures were modified to test the effect of smoke solutions have on seed germination under laboratory conditions and seedling emergence under nursery conditions.

3.1.3 Seed germination chambers

Two custom built germination chambers were used for the laboratory trial (Plate 3.1). The chambers were set to maintain a constant temperature of 25ºC in the presence of fluorescent lighting.

Plate 3.1 Petri dishes in a germination chamber

3.1.4 Nursery conditions

Both Sappi's research nursery at Tweedie and Sappi's commercial nursery at Richmond were used for trials in this study. The design and operation of both nurseries are very similar (Plate 3.2). Placing trials under nursery conditions was done in order that any beneficial treatment identified could be utilised under similar commercial growing conditions. As there are no direct comparisons drawn between the two trials the use of different nurseries did not affect the accuracy of the trials.

In both nurseries trays were placed on rails supported by a wooden sub-structure. The trays were raised about 1 m from the ground which reduced the risk of contamination from soil-borne, or surface water-borne disease organisms. A plastic roof prevented rain water from reaching the trays. This environmental protection ensures that seeds are not over-watered. The nursery trays were irrigated using a moving boom which travels at a constant speed providing a uniform application of water. Irrigation scheduling was determined by the standard commercial nursery management practice.

Plate 3.2 The nursery structure showing experimental seedling trays

3.1.5 The nursery trays used

The nursery trays used were the “Clausen” 49-cavity black plastic trays routinely used by Sappi in their commercial nurseries (Plate 3.3). Prior to sowing, the trays were cleaned using a high pressure washing spray. This was done in order to reduce the chances of disease contamination.

Plate 3.3 The detail of the nursery trays used

3.1.6 The bark medium

The bark medium used in the nursery trials was from the commercial source being used by Richmond nursery at the time of the trial. The bark is assumed to have provided a uniform growing medium. No problems were experienced raising other commercial seedlings in bark of the same batch at the time of the trial. Bark of this quality would thus be routinely used.

3.1.7 The smoke generation equipment and the preparation of the smoke solution

The equipment used to generate the smoke solution was a custom-made furnace with a water-cooled exhaust pipe. The door to the combustion chamber was closed once the fire was well established. Smoke was drawn from the furnace and propelled through a 200ℓ plastic water drum by the action of a commercial spray gun powered by an industrial air compressor (Plate 3.4). Literature available at the time indicated that a variety of pieces of equipment were being used by different research programmes and it was not important to create the smoke solution in accordance with any set standard. The design was not copied from any other source but built from available material. The plant was successful in creating a concentrated smoke solution. The smoke was generated by burning pine cones, wood and damp cardboard. Smoke was pumped through the water in the drum for 2h until a dark solution had been created. The drum was then sealed and shaken in order to create a uniform mix.

The resultant smoke solution was then decanted by siphoning it into *5ℓ* brown glass bottles. The bottles had been thoroughly cleaned with soap and hot water, and oven-dried to ensure they carried no contaminants. No information was available on storing smoke solutions. Brown glass bottles should have prevented any detrimental affect light may have, while glass would ensure that no reaction with the container was likely. The bottles of smoke solution were stored in a cold-room, used for seed storage which is kept at 6ºC. This

concentrated smoke solution formed the base used in all the tests. All the smoke solution used was taken from this one 200ℓ batch and diluted in a 1:4 ratio with tap water.

Plate 3.4 The equipment constructed and used to generate the smoke solution

3.1.8 The concentration of the smoke solution

From the literature available there was no way of determining what would constitute the ideal concentration of a smoke solution. The effect of concentration of the smoke solution was specifically delimited from the study. It is known that rinsing seed reduces any inhibitory effects (while not removing stimulatory effects) caused by the application of smoke (Brown. 1993a). Thus inclusion of rinsing as a treatment (whenever a smoke solution was used) was assumed to be effective in negating any detrimental effects of

concentration. As smoke solutions are known to be effective at very low concentrations (1:50000) (Brown. 1993a) it was assumed the solution could not be too weak to achieve the desired effect.

3.2 Methods

3.2.1 A brief description of the various standard seed pre-treatments

Hydropriming: In this study, the term "hydropriming" is used in place of the term "imbibed" which is used in the Sappi Forests Research Nursery Manual (2004). The term "hydropriming" is more widely used and more applicable in describing the process. Hydropriming involves placing the seed in de-ionized water that is constantly aerated and held at a constant temperature of 25ºC.

Target Moisture Stratification (TMS): The specific aim of the target moisture stratification pre-treatment is to allow the seed to overcome dormancy by stratifying seed that has a moisture content of 32% for more than 28 days. The amount of moisture needed to reach this target percentage is calculated according to the formula contained in the Sappi Forests Research Nursery Manual (2004). The required moisture is placed with the seed in a plastic bag and the bag is then sealed. The seed imbibes the given moisture while being refrigerated at 6ºC. The moisture was supplied in the form of either de-ionized water, or in the form of the smoke solution.

Pre-germination: In this study the term "Pre-Germ" refers to the specific technique as in pine use. The pre-germination treatment is designed to soften the seed coat while the seed is imbibing water. This is achieved by placing the seed in a 1% solution of hydrogen peroxide held at 25ºC for 4 days while being constantly aerated.

3.2.2 Trial 1: A pilot study to determine the sample size required

The aim of this experiment was to establish the amount of variance found to occur in the untreated base seed sources of *P. elliottii* and *P. taeda.* To test this variation under both controlled and nursery conditions in order to establish the experimental unit size needed to detect significant differences at a 95% confidence level, was determined.

Trial design and method:
Petri dishes (50) containing 25 seeds per dish were prepared for both species from the base seed lots. The seed were given no pre-treatment and tested according to standard Sappi seed testing protocol for dry sown seed in a controlled environment. The number of seeds that germinated after 30 days in each dish was recorded.

Fifty nursery trays (49 seeds per tray) were prepared and sown with one seed per cavity. The seeds were given no pre-treatment and tested according to standard Sappi seed testing protocol for dry sown seed in a nursery environment. The number of seeds that germinated in each tray after 42 days was recorded.

Statistical analysis: The sample size required to determine significance was calculated using the following formula:

$$N = (t^2 X S^2)/ d^2$$

Where:

N = minimum no. of samples required

t = t-value for standard level of confidence i.e. (95% confidence)

S^2 sample variance estimated from pilot trial

d = specified difference that would be biologically meaningful (e.g. within 5% of the expected mean)

3.2.3 Trial 2: Effect of smoke treatments on seed germination under controlled laboratory conditions

The primary aim of this experiment was to determine if subjecting seed of both *P. elliottii* and *P. taeda* to three standard pre-treatments, namely hydropriming, pre-germination and TMS, in combination with water, a smoke solution and a smoke and rinsing treatment (for those exposed to the smoke solution), would result in any significant increase in the germination percentage under controlled conditions. Dry-sown seed was included as a control. The secondary aim of this trial was to use the results to identify those treatments which gave no response or a negative response. Non-responsive treatments would not be carried forward in Trial 3 in order to make the trial more manageable.

Trial design and method: Three germination stimulant treatments were tested at three levels of smoke solution, namely no smoke (water only), smoke solution, and smoke solution and rinse. Pre-germination and TMS were also used in combination with smoke treatment (Table 3.1).

Table 3.1 Treatment combinations for laboratory test of seed performance

	Treatments									
	Water	Smoke solution	Smoke solution/ rinsed	Pre-germinated in water only	Pre-germinated with smoke solution	Pre-germinated with smoke solution and rinse	TMS seeds treated with water only	TMS seeds treated with smoke solution	TMS seeds treated with smoke solution and rinsed	Dry sown seeds
P. elliottii	25*	25	25	25	25	25	25	25	25	25
P. taeda	25	25	25	25	25	25	25	25	25	25

* 25 = the number of replications for each treatment.

All seed pre-treatments were scheduled so that sowing of the seed into the Petri dishes occurred on the same day. Rinsing was achieved by placing the seed in net bags and placing them under running water for 1 h.

The temperature was set at 25ºC and the seed given 16 hours of light and 8 hours of dark. Percentage germination was determined after 28 days. The trials were analyzed seperatly. Radicle emergence was taken as an initial indication of germination. Seedlings which developed abnormally were excluded from the score at the end of the monitoring period. On the emergence of the radicle, the seed was placed in a separate Petri dish in order to be able to score daily emergence. Each day, after monitoring, the Petri dishes were moved randomly on the shelves of the growth chambers to eliminate any effect of placement within the chamber.

Statistical analysis: Data were subjected to generalised analysis of variance (ANOVA) using GENSTAT (version 9). To determine differences between treatment means, LSD (least significant difference) and SED (standard error of the difference) were used.

3.2.4 Trial 3: Effect of smoke treatments on seedling establishment under nursery conditions

From the initial analysis of Trial 2, interesting treatment by species interactions was noted. It was decided that none of the treatments from Trial 2 would be excluded in the design of Trial 3. Despite the size and resources needed to carry all the treatments forward, all the combinations were carried out under nursery conditions. In addition a further media treatment was added. Seeds were sown in media composed of pine bark treated at three levels of smoke solution impregnation.

Trial design and method: The trial included all the treatments at the same three levels of smoke solution for the same species as described in Trial 2. In addition, Trial 3 included an additional treatment, namely media impregnation with smoke solution at three levels. The levels were:

a) No impregnation.

b) Impregnation with standard smoke solution.

c) Impregnation with standard smoke solution followed by 4 applications of a booster dose of standard smoke solution (Table 3.2).

Table 3.2 Treatment combinations for tests under nursery conditions (Trial 3).

	Species	**Standard Germination Stimulation**	**Smoke Solution Application**	**Media**	
	P. taeda	Hydro-primed	None	Not impregnated with smoke solution	
	P. elliottii	Pre-germinated	Standard solution	Impregnated with smoke solution	
		Target moisture stratified	Standard solution and rinse	Impregnated with smoke solution and boosted.	
Number of combinations	2	3	3	3	54
The dry-sown control was sown for each species and into the 3 media levels					6
Total No. of combinations					60

Sufficient seed to sow 12 replications of 49 seeds per tray per species for each treatment, at 3 media levels were set aside. Sufficient dry sown seed for each species was set aside to act as a control. The controls were to be sown in each level of the media treatment. The seed pre-treatments were scheduled so that all the seed was ready to be sown on the same day.

The trial was designed as a randomised complete block design incorporating a split block layout. The split plots were useful in identifying and applying smoke solution to those

treatments levels that required further applications. Twelve replications were incorporated in the design. This design was in accordance with the estimate of variance determined from Trial 1. The trial design specifically aimed to address the effect of any environmental gradient that may be experienced in placing a trial of this type in a nursery. Half the replications were placed on the eastern side of a nursery table and half on the western side as only one nursery table was available for the trial.

A single row of seedling trays formed a boundary row around the perimeter of the trial. No seed was sown in the trays used to form boundary rows as these trays needed to be moved daily while recording emergence. The presence or absence of emerging seedlings in the boundary rows would have had little affect on the trial (Plate 3.2)

The trays were filled with media (Plate 3.5) and each tray individually labelled prior to sowing the trial. The trays were filled with normal composted pine bark or smoke-impregnated pine bark according to the trial design. Fine sieved bark was prepared for capping the seed. Prior to filling the trays with the smoke-impregnated bark, the bark was thoroughly watered with smoke solution of the standard concentration, mixed by hand with a spade, and left overnight for any excess smoke solution to drain away. Although the bark medium was moist before the smoke solution was added it was apparent that the bark did absorb some of the smoke solution and some remained on the bark surface. The finer bark medium used for capping the sown seed was also impregnated with smoke solution for use on those trays requiring smoke-treated bark media according to the trial design.

Plate 3.5 Filling of the seedling trays and hand-sowing for Trial 3.

For the media treatment requiring further doses of smoke solution these were applied at weekly intervals for 4 weeks, one week after the trial had been laid out in the nursery. The solution was applied using a knapsack and a dosing gun (Plate 3.6). Each cavity received 20 ml of solution per week. Applying additional doses of solution was done to counter the effects of leaching and to afford the emerging seedlings additional treatment with smoke-solution.

Plate 3.6 Application of smoke solution as a booster treatment.

The seeds were sown by hand and the trays capped with fine-sieved bark which had received the appropriate treatment. Each replication was sown separately and in order. The trays were then placed in the nursery according to the trial lay-out design. Each replication was placed separately and in order.

Trial 3 was placed in the nursery on 11 March 2006 and monitored for 45 days. From prior experience Sappi's commercial nurseries would consider this an acceptable time of the year to be sowing these species of pine seed. The trial was watered daily as would occur in normal commercial practice. No records of the nursery climate were kept.

For the first 8 days after sowing the trial no recording of emergence was undertaken, as it was known from experience that it is unlikely any seed would have emerged in this early period. The trial was monitored daily for the next 37 days. The number of seedlings emerging on any tray was recorded daily. Every seedling that emerged was marked by inserting a toothpick in the cavity where the seedling emerged.

Once germination monitoring had been completed, root and shoot growth was measured. The sum of the dry mass of all the roots and then all the shoots from each individual tray were recorded.

To determine the mass of the roots, each seedling that had emerged was removed from the tray and the roots were washed to remove all the bark compost (Plate 3.7). All the roots collected from one tray were then placed in a paper bag. All the moisture was removed by drying the bags containing the roots in a drying oven set at 75ºC for three days. The contents of bags were then weighed and the values recorded. All the shoots from each tray were placed in a separate bag and dried in the same way as the roots. The contents of bags were then weighed. The dry mass of the roots or the shoots was adjusted to account for different emergence rates by dividing the mass by the number of seeds that had emerged from each individual tray.

Plate 3.7 Seedling preparation before determination of growth parameters

3.2.5 Statistical analysis

Data was subjected to generalised analysis of variance (ANOVA) using GENSTAT (version 9). To determine differences between treatment means, LSD (least significant difference) and SED (standard error of the difference) were used.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Trial 1: Determination of optimal conditions for experimentation

This pilot study was conducted to determine the experimental unit size needed to detect significant differences at a 95% confidence level (see Section 3.2.2). This was done for both *P. elloittii* and *P. taeda* and also for both Petri dish and nursery conditions using the formula given in Section 3.3.2. The results showed that *P. elliottii* needed 21.912 seeds and *P. taeda* needed 26.127 seeds per Petri dish. These numbers of seeds would give a sufficiently large enough sample for statistical purposes under laboratory conditions. *P. taeda* ideally required 26.127 seeds but it was felt that 25 seeds per Petri dish was close enough for statistical purposes as 25 seeds per Petri dish is the number prescribed for routine testing.

The pilot study showed that for the nursery conditions *P. elliottii* needed an average of 39.860 seeds per tray and *P. taeda* needed 43.776 seeds. As a standard Sappi nursery tray has 49 cavities the results showed that 49 seeds was an adequate experimental unit size.

4.2 Trial 2: Seed germination

P. taeda and *P. elliottii* were placed in different growth chambers as there was insufficient space for both species in one growth chamber. Percentage germination was determined after 28 days. The trials were thus analyzed separately.

In presenting the results the untransformed data were used for the tables, discussions, and graphs of the results while the arcsine transformed data were used to determine significance of differences.

4.2.1 Germination of *P. elliottii*

The method of seed pre-treatment had the most significant effect ($p < 0.001$). The interactions of the smoke solutions were not significant ($p < 0.454$). Thus additions of smoke did not increase germination in Petri dish under laboratory conditions for this species. The full set of results of the analysis variance of the *P. elliottii* germination data are attached as Appendix 1. Figure 4.1 below, derived from the table of means, illustrates the effects of different seed treatments on germination.

The Pre-germ method resulted in the best germination of 32.91±1.384%. Although the comparisons were made on arcsine transformed data the results in figure 4.1 and the following discussion is based on the actual means from the untransformed data.

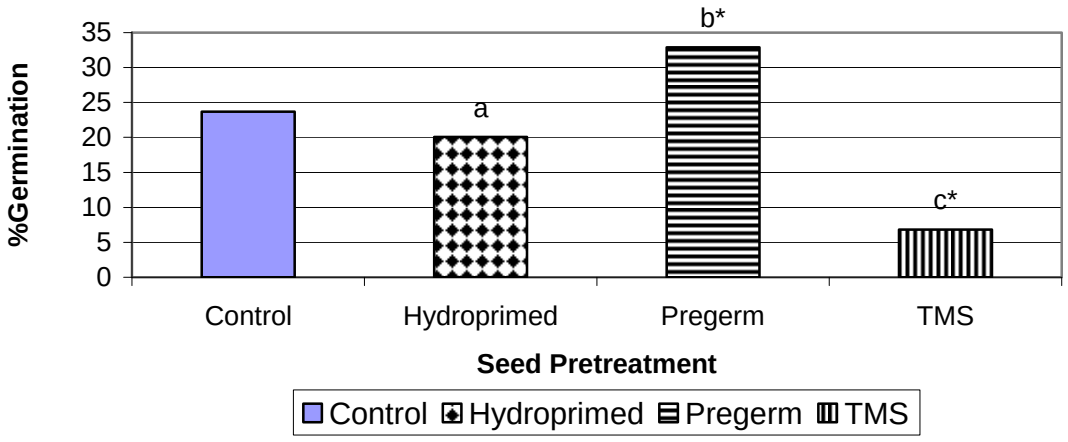

Figure 4.1 Effect of different seed treatments on germination of *P. elliottii*.
Treatments with similar letters are not significantly different (LSD (0.005) = 4.110).
Treatments marked with an asterisk are significantly different from the control.

From Figure 4.1 it is clear that the pre-germ treatment gave the best germination (mean: 32.91±1.38%) significantly better ($p < 0.005$) than the control (23.68±2.63%) and the hydroprimed treatment (20.05±1.19%). The TMS treatment gave the significantly ($p <$

0.005), and notably, the poorest germination (6.83±0.88%). This was 16.85% less than the control. The control (23.68±2.68%) performed better than the hydroprimed treatment (20.05±1.19%) though the difference was not significant ($p < 0.005$). The interactions where the seed was treated with smoke solution, and smoke solution and rinsed, had no significant effects.

The main aim of study was to specifically examine whether the addition of smoke, or smoke and rinse treatments in combination with existing standard pre-treatment methods used on seed would result in a beneficial gain of over 5%. Accordingly, it can be reported that addition of smoke, or smoke and rinse, treatments to *P. elliottii* seed, of this seed lot, cannot be shown to have increased the germination in Petri dishes by 5%.

4.2.2 Germination of *P. taeda*

The method of seed pre-treatment was significant ($p < 0.001$) and the effect of smoke significant ($p < 0.013$) and the interaction of smoke and seed pre-treatment method was the most significant ($p < 0.001$) interaction. The full set of results of the analysis variance of the *P. taeda* germination data is attached as Appendix 2. Figure 4.2 below illustrates the results. The best results were obtained from the interaction of seed having been pre-treated with the TMS method and being exposed to a smoke solution and rinsed (84.32±2.12%). This treatment was significantly better ($p < 0.005$), (LSD = 4.649) than the next best result treatment, namely TMS treated seed with water (73.60±1.74%). The TMS seed treated with smoke gave the third best result (72.48±2.21%) which was not significantly different ($p < .005$) from the TMS, (seed with water), treatment. All three treatments using TMS as a pre-treatment were significantly better ($p < .005$) than the control (62.72±2.43%). Although the comparisons were made on arcsine transformed data, the results in Figure 4.2. present the means from the untransformed data.

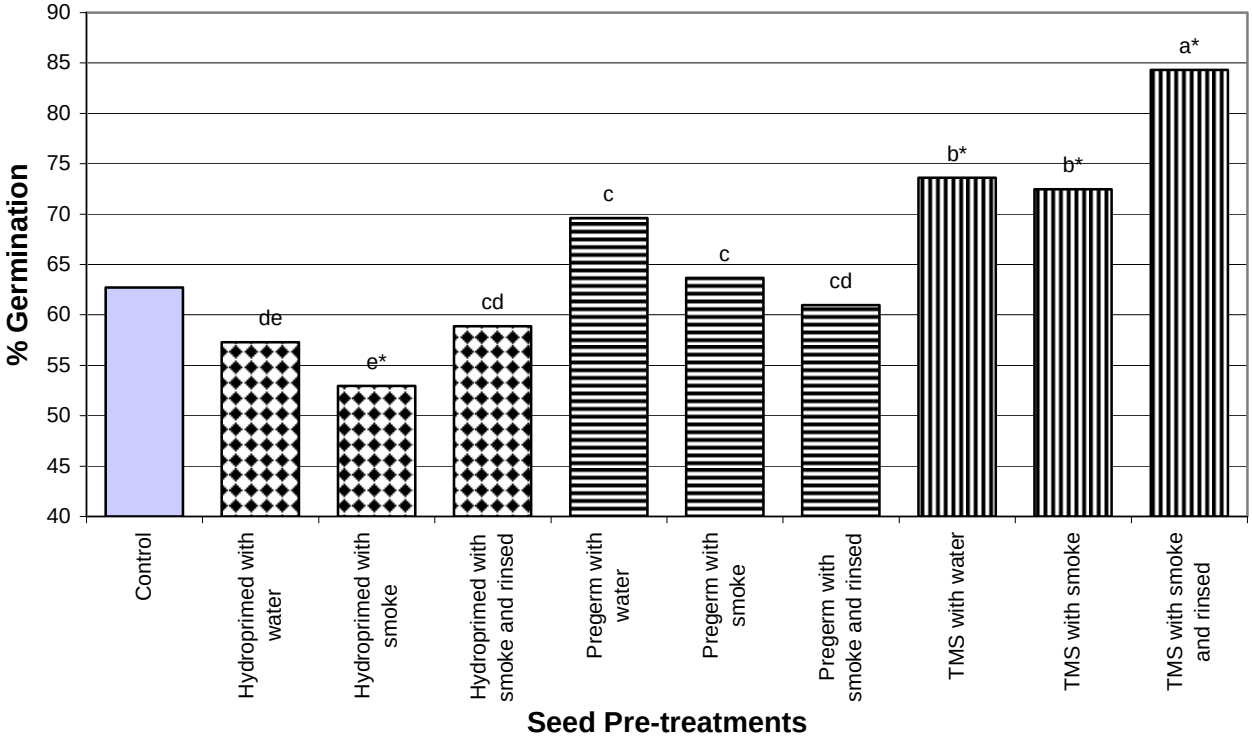

Figure 4.2 Effect of treatments on germination of *P. taeda* seeds.
Treatments with similar letters are not significantly different (LSD (0.005) = 0.649).
Treatments marked with an asterisk are significantly different from the control.

Seed treated with TMS performed significantly ($p < 0.005$) better than seed treated with the pre-germ method. The Pre-germ with water treatment gave 69.60±2.14%. The Pre-germ with smoke treatment gave 63.68±2.62%. The Pre-germ with smoke and rinse treatment gave 60.96±2.73%. There were however no significant differences ($p < 0.005$) between the three Pre-germ treatments. None of the pre-germ methods were significantly different from the control (62.72±2.73%)(Figure 4.2).

The hydroprimed pre-treatment gave the worst result of the three seed pre-treatments. Of the three hydroprimed treatments, seed hydroprimed with smoke and rinse gave the best result (58.88±2.89%). This germination was not significantly better than the worst Pre-

germ treatment, namely Pre-germ smoke and rinse. The seed hydroprimed with water gave 57.28±2.58% germination. Seed hydroprimed with water, and seed hydroprimed with smoke solution and rinsed, were not significantly ($p < 0.005$) different from each other and from the control. Seed hydroprimed with smoke gave 52.96±2.812%. This result was significantly ($p < 0.005$) lower than the control (62.72±2.43%) but not significantly ($p < 0.005$) different from the seed hydroprimed with water only (Figure 4.2).

All the TMS seed pre-treatments gave significantly ($P< 0.005$) better results than the other seed pre-treatments. The fact that the TMS seed treated with water responded slightly better (1.12%) than the TMS seed treated with smoke could indicate that the smoke solution was having a small, but not significant ($p < 0.005$), inhibitory effect. The addition of a rinse increased the germination by 11.84% over the smoke treated TMS seed. With an LSD of 4.649, an 11.84 % increase in germination would indicate this combination of seed pretreatment and smoke and rinse was giving a minimum increase in germination of 7.19% (Figure 4.2).

Baldwin et al. (1994) and Brown (1993a) showed that rinsing may remove the inhibitory effects that the smoke solution may have, due to being at too high a concentration, while not removing the stimulatory effect. The inclusion of a rinsing treatment may explain why this combination responded better than the other TMS treatments.

The seed Pre-germed with water (69.60±2.14%) performed better than the seed pre-germinated with smoke (63.68±2.62%), and the seed pre-germed with smoke and rinsed (60.96±2.73%). Though none of these treatments were significantly different ($p < 0.005$) to the control, the addition of smoke, and smoke and rinse, are clearly not beneficial. Seed Pre-germed with water performed significantly better ($p < 0.005$) than seed Pre-germed with smoke and rinse (Figure 4.2).

The main aim of this study, which was to specifically examine whether the addition of smoke or smoke and rinse treatments in combination with existing standard pre-treatment methods used on seed would yield a beneficial effect of over 5%. It can be reported that addition of smoke and rinse in combination with the TMS method of seed pre-treatments to *P. taeda* seed of this seed lot increased the germination in under laboratory conditions by 5%.

4.2.3 Comparison between the results of the germination of *P. elliottii* and *P. taeda*

What is of interest is that the two species responded differently to the same seed pre-treatments. The two species responded differently to the inclusion of a smoke, and a smoke and rinse treatment. The seeds *P. elliottii* did not show any significant response to the inclusion of these treatments while in comparison the seeds of *P. taeda* did. (Figures 4.1 and 4.2). *P. elliottii* seed treated with the pre-germ and hydroprimed method responded significantly better ($p < 0.005$) than the control. The hydroprimed, pre-germed and control gave a significantly better ($0 < 005$) response than the TMS method. (Figure 4.1).

In comparison to the control, the *P. taeda* gave a significantly better response to the TMS method while not responding significantly differently from the control for the pre-germ and hydroprimed method. Except in the case of seed hydroprimed with water where the control performed significantly better ($p < 0.005$) (Figure 4.2).

These results indicate that different species respond differently to the pre- treatments. In *P. elliottii* there was no significant benefit of introducing smoke to the existing pre-treatment while in *P. taeda*, combining the TMS pretreatment with a smoke and rinse treatment significantly increased germination. (Figures 4.1 and 4.2).

The main aim of this study was to examine whether the addition of smoke, or smoke and rinse treatments, in combination with existing standard treatment methods, could yield a

5% improvement in seed germination. In accordance with this aim it can be reported that treating *P. taeda* seeds with a smoke and rinse treatment in combination with the TMS pre-treatment method a 5% increase in germination can be obtained.

4.3 Trial 3: Nursery emergence

Trial 3 differs from Trial 2 in that seed of the same species and seed lots subject to the same pretreatments were sown in nursery conditions. In addition to the combination of treatments used in Trial 2 three different media were used in the nursery trials. In reporting on the results of Trial 3 the results of the two species will be considered separately. This is done as in practice different species would not be mixed together in a commercial operation. The results of the different treatments on seedling growth will also be presented and discussed separately by species. If a treatment did not enhance emergence significantly, the effect of that treatment on subsequent growth will not be examined. In the commercial operations trial any treatment resulting in increased emergence would be considered more beneficial than the effects of subsequent seedling growth.

4.3.1 Emergence of *P. taeda* in the nursery

The seed pre-treatment was the only significant effect ($p < 0.001$) (Figure 4.3). The addition of smoke in any form had no significant ($p < 0.001$) effect on the emergence of the *P. taeda* seed in the nursery. The interaction between the pre-treatments and the smoke treatments were not significant ($p < 0.799$). The interactions between the pre-treatments, the smoke treatments and the media treatments were also not significant ($p < 0.557$).

These findings are surprising given that in Trial 2 *P. taeda* responded significantly ($p < 0.005$) and positively to the interaction with the TMS smoke and rinse interaction (Figure 4.2). The full set of results of the analysis variance of the *P. taeda* emergence data is attached as Appendix 3. Figure 4.3 which is derived from the table of means, illustrates the results. Although the comparisons were made on arcsine transformed data the results in

Figure 4.3 and the following are based discussion is based on the actual means from the untransformed data.

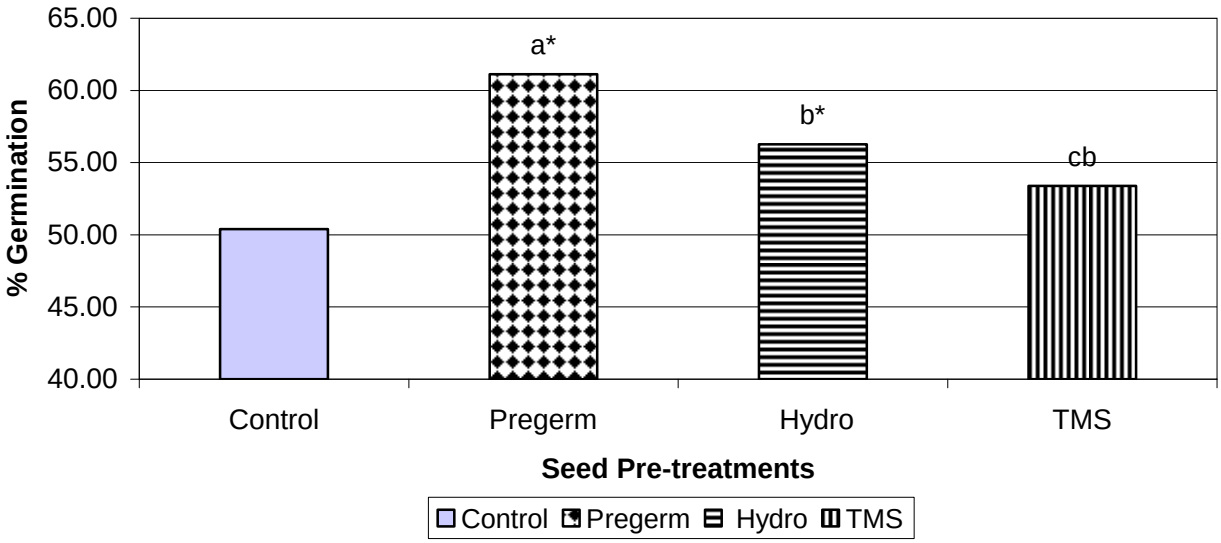

Figure 4.3 Emergence of *P. taeda* under nursery conditions.
Treatments with similar letters are not significantly ($p < 0.005$) LSD = 2.202) different.
Treatments marked with an asterisk are significantly ($p < 0.005$) different from the control.
The control mean was 50.40±0.005%.

The pre-germ method gave the best result (61.13±2.63%) which was significantly better ($p < 0.005$) than the hydroprimed method (56.27±2.54%) and the control (50.40±1.19%). The pre-germ method was 10.73% better than the control. The LSD for the trial was 2.202 (Figure 4.3). The TMS method resulted in 53.38±2.202% emergence, significantly poorer than the pre-germ method but not significantly ($p < 0.005$) poorer than the hydroprimed method or than the control (Figure 4.3).

In accordance with the main aim of this trial which was to see if the addition of smoke would result in a 5% increase of seed emergence, it is concluded that smoke in combination with the existing pre-treatments did not yield any discernable improvement in the emergence of *P. taeda* seed (of this seed lot.).

4.3.2 Comparison of germination trial with nursery trial for *P. taeda*

The most notable result was that the best combination of treatments in Trial 2 did not produce the best results in Trial 3. In Trial 2 the interaction of the smoke and pre-treatments produced significant ($p < 0.005$) results. The same seed subjected to the same pre-treatments sown into bark media at the different levels of smoke impregnation, placed in the nursery produced no significant ($p < 0.533$) interactions. See Figures 4.2. and 4.3. In Trial 2 the TMS method gave the significantly ($p < 0.005$) best germination results, within the trial, for all three smoke treatments. In Trial 3 the TMS method gave the significantly ($p < 0.005$) worst treatment result. In Trial 2 the pre-germ method gave the second best set of results whereas in Trial 3 this method gave the best set of results (Figures 4.2 and 4.3). The hydroprimed method which gave the worst results in Trial 2 gave the second best results in Trial 3. There does not appear to be any noticeable trends between the results of the two trials.

The different germination results obtained from germinating seed in a laboratory environment to those obtained by sowing the same seed (having undergone the same pretreatments and additions of smoke) in a nursery environment is remarkable. There was no indication in the literature that such inconsistent results between the Petri dish and nursery environment could be expected. None of the work cited in the literature review reported having tested the germination under different conditions to see if the results were consistent. The tests were either done in Petri dish, nursery or field environments.

The reason that seed reacted so differently in different environments is interesting and may provide an interesting avenue for further study. The main reason for testing the seed in

nursery conditions was to test if results obtained in Petri dish conditions were expressed under nursery conditions with a view to being able to commercially exploit any positive gains.

The main aim of this thesis was to see if any combination of the proposed treatments could yield a 5% increase in seed recovery. Trial 3 demonstrated that the increased germination obtained by using a combination of smoke, rinse and TMS treatments in Petri dishes is not repeatable in a nursery environment. It is beyond the scope of this study to try and discover why the seed reacted so differently in different environments, but merely to record that it did.

4.3.3 Emergence of *P. elliottii* in the nursery

The interactions between the pre-treatment method, the smoke treatments, the media and the control were significant ($p < 0.044$). The full table of results is included as Appendix 4.

In Trial 3 there were three different seed pre-treatments, three different smoke application treatments, sown into three different media and as a result there are too many resulting combinations to discuss each result. As a consequence only the best result, and those results not significantly ($p < 0.005$) different to the best result, will be considered. These results were all significantly ($p < 0.005$) better than the control (Figure 4.3.2). Although the comparisons were made on arcsine transformed data the results in Figure 4.4 and the following discussion is based on the actual means from the untransformed data.

The best result was obtained was obtained from seed Pregermed in water and sown into plain bark (40.65±2.63%). This finding is notable in that this combination of treatments outperformed any of the treatments involving smoke in some combination. The LSD of this trial was 4.622. Although the best result, this result was not significantly ($p < 0.005$) different to the results of five other treatments listed below (Figure 4.4).

- Seed hydroprimed with smoke and rinse solution, planted in impregnated bark media with additional smoke water additions (36.90±1.94%, Figure 4.4).

- Seed hydroprimed with smoke solution, planted in impregnated bark media with additional smoke water additions (36.90±3.32%, Figure 4.4).

- Seed hydroprimed with water, planted in impregnated bark media with additional smoke water additions (36.56±1.9%, Figure 4.4).

- Seed pre-germed with smoke solution and planted in plain bark (36.22±2.04%, Figure 4.4).

- Seed pre-germed with water solution, planted in impregnated bark media with additional smoke water additions. (36.22±1.687%, Figure 4.4).

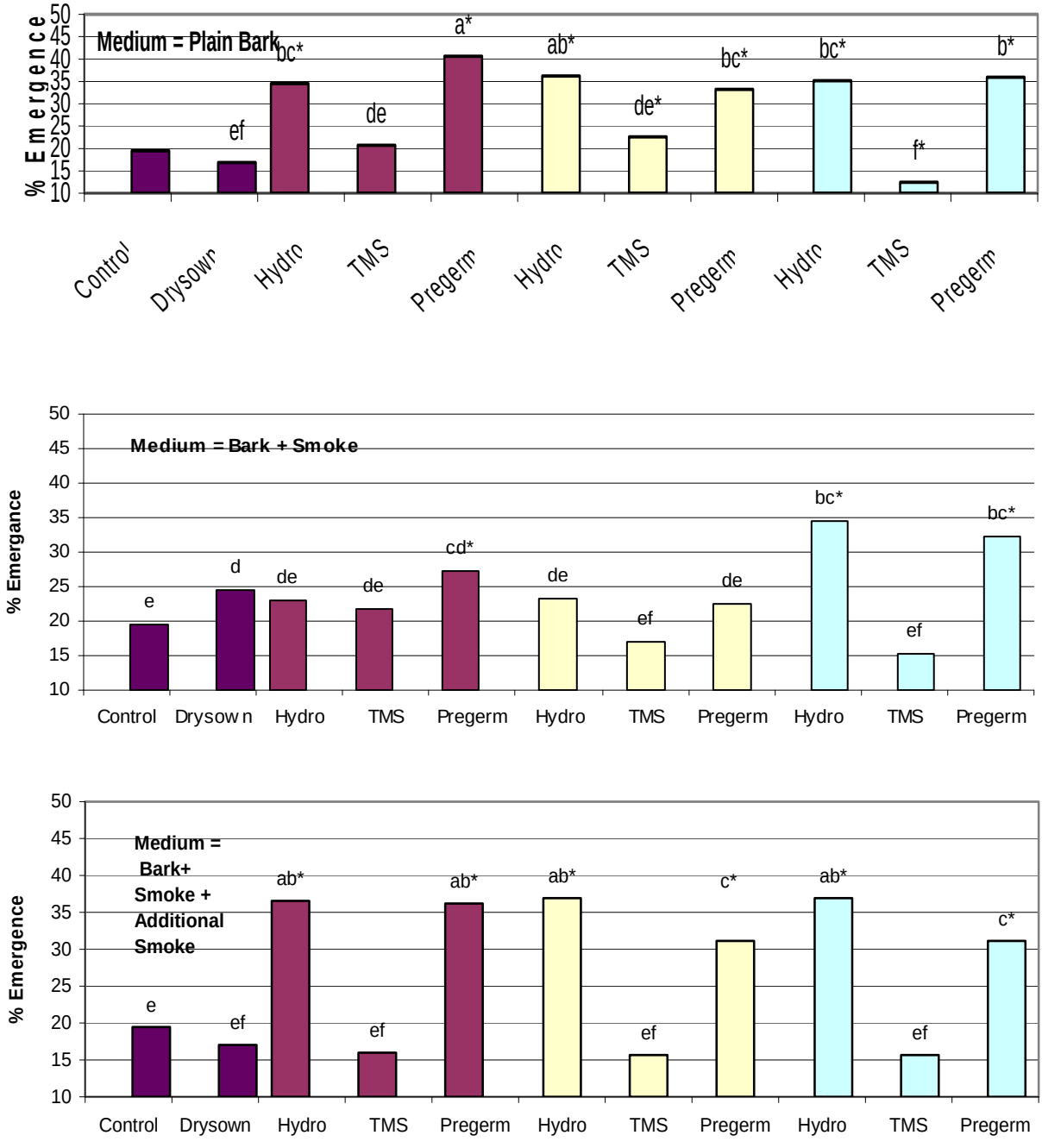

Figure 4.4. Emergence of *P. elliottii* in response to various seed treatments. Treatments with similar letters are not significantly different (LSD ($p < 0.005$) = 4.622). Treatments marked with an asterisk are significantly different from the control.

In considering the best six results, that were not significantly different from each, the following can be noted:

- The best treatment was obtained from seed pre-germinated in water and planted into plain bark.

- All six best treatments came from the hydro-priming and pre-germination seed pre-treatments. There was no overall significantly different ($p < 0.005$) results between these two seed pre treatment methods, among the six best results.

- Smoke (whether present or absent) in the seed pre-treatment or the media appeared to play no role in determining the best six results

The following trends are noted:

- All the TMS treatments performed poorly in relation to the other pre-treatments, they were however not significantly ($p < 0.005$) better or worse than the control except one (seed treated with TMS smoke and rinse and planted in plain bark which was significantly worse than the control). (Figure 4.4)

4.3.4 Comparison of germination trial with nursery trial for *P. elliottii*

In Trial 2 the pre-germ method gave the best results (Figure 4.1). In Trial 3 although the interactions at all levels was significant ($P < 0.044$) the pre-germ seed, exposed to water only gave the best result of the trial when planted in plain bark (Figure 4.4). One of the five best results, (that were not significantly different to the best result ($p < 0.005$) was seed pre-germed in water and planted in the smoke impregnated media that received additional smoke treatments (Figure 4.3).

In Trial 2 the hydroprimed seed performed significantly worse than the pre-germ treated seed but still significantly ($p < 0.005$) better than the control (Figure 4.1). In Trial 3 this trend is repeated though the difference in four of the pre-germ treatments is not significantly ($p < 0.005$) poorer than the hydroprimed seed which did the best (Figures 4.1 and 4.3).

In both Trials 2 and Trial 3 the TMS treatment gave the worst result though, except in one case in Trial 3, these results were not significantly worse than the control (Figures 4.1 and 4.2).

The aim of this study was to see if any treatment or combination of treatments involving smoke would result in a 5% increase of seed recovery. Accordingly it can be reported that germinating *P. elliottii* seed (of this seed lot) in Petri dishes, smoke in combination with the other standard seed pre-treatments played no significant role in promoting germination.

4.4 Seedling growth

4.4.1 *P. elliottii*

The most significant effect on early growth was the pre-treatment method ($p < 001$) LSD = 0.11817). The interactions between method, media and smoke treatments was significant ($p < 0.002$) but not as significant as the pre-treatment. The full set of results from the analysis is included as Appendix 5. The results presented are taken from transformed data and standard errors are included. Seed pre-germinated with water planted in bark impregnated with smoke that received additional smoke treatments performed the best (0.8131±0.006 g LSD = 0.11871). This result was significantly ($p < 0.005$) better than the control (Figure 4.4.1). The results presented represent the combined mass of the roots and shoots.

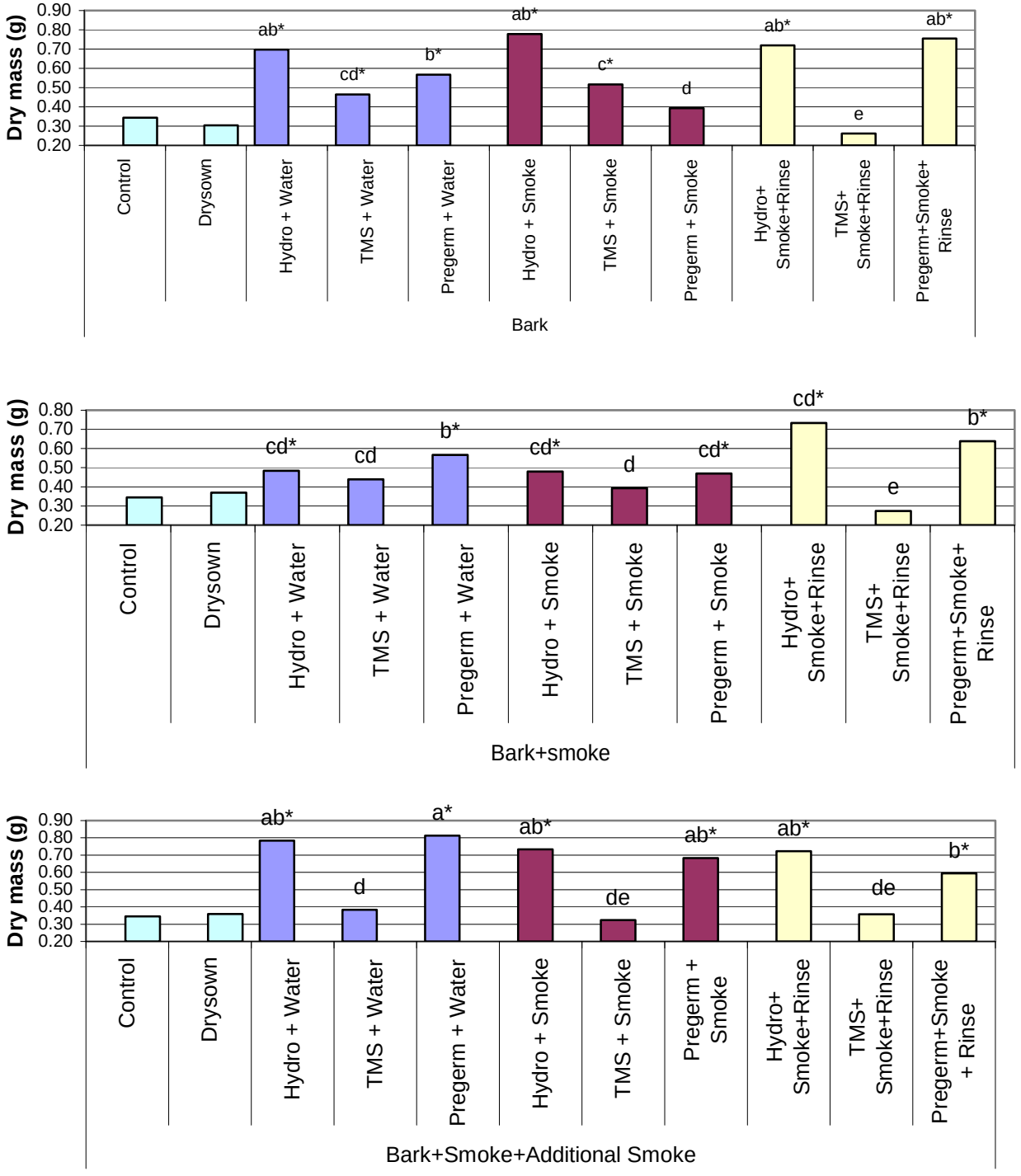

Figure 4.5. Mean dry mass of *P. elliottii* seedlings in response to various seed treatments. Treatments marked with an asterisk are significantly different from the control. The trial mean across all three media was 0.5445 (Figure 4.5).

Early growth was significantly better than the control (0.3445±0.005 g) in all the following eight best, treatment combinations. None of the eight are significantly ($p < 0.005$) different from each other (Figure 4.5). The eight best treatments were:

- Seed hydroprimed with water planted in plain bark media (0.6969±0.00793 g).
- Seed hydroprimed with smoke planted in plain bark media (0.7833±0.00787 g).
- Seed hydroprimed with smoke and rinse planted in plain bark media (0.7129±0.00631 g).
- Seed pre-germed in smoke and rinsed planted in plain bark media (0.7552±0.00505 g).
- Seed hydroprimed with smoke and rinsed planted in bark media impregnated with smoke (0.7336±0.00630 g).
- Seed hydroprimed with water planted in bark media impregnated with smoke that received additional smoke treatments (0.7832± 0.00523 g).
- Seed pre-germed in water planted in bark media impregnated with smoke that received additional smoke treatments (0.8131± 0.00677g).

The following observations were made:

- Both pre-germination and hydropriming seed pre-treatment methods resulted in some of the eight best treatments. All the TMS treatments gave significantly poorer ($p < 0.005$) results.

- From the emergence data from Trial 3 both pre-germination and hydropriming treatments gave the five best results that were not significantly ($p < 0.005$) different from each other.

- Seed exposed to water, smoke, smoke and rinse treatments, as well as these combinations being planted in all three bark media are represented in the eight best results

It is thus concluded that the addition of smoke in any combination had no discernable benefit in the early plant growth of *P. elliottii.* The best early growth occurred where no smoke was present.

4.4.2 P. *taeda*

The method of seed pre-treatment had the most significant ($p < 0.001$) effect of the early growth of the seedlings, and is not included in Figure 4.4. Thus the addition of smoke treatments resulted in no significant ($p < 0.001$) early growth of the seedlings. The LSD for the analysis was 0.05982 g. The results are taken from untransformed data. The full set of results from the analysis is attached as Appendix 6

- The TMS treatment gave the best growth response (0.8629±0.00631 g) which was significantly ($p < 0.005$) better than the control.
- The pre-germ treatment gave the second best growth response (0.7483±0.00706 g) which was significantly ($p < 0.005$) poorer than the TMS treatment but still significantly ($p < 0.005$) better than the control (Figure 4.6). The mean for the control was 0.5101±0.00451 g.
- The hydroprimed treatment gave the third best response (0.6820±0.00433 g) which was significantly ($p < 0.005$) poorer than the pre-germ and TMS treatment but still significantly ($p < 0.005$) better than the control (Figure 4.6).

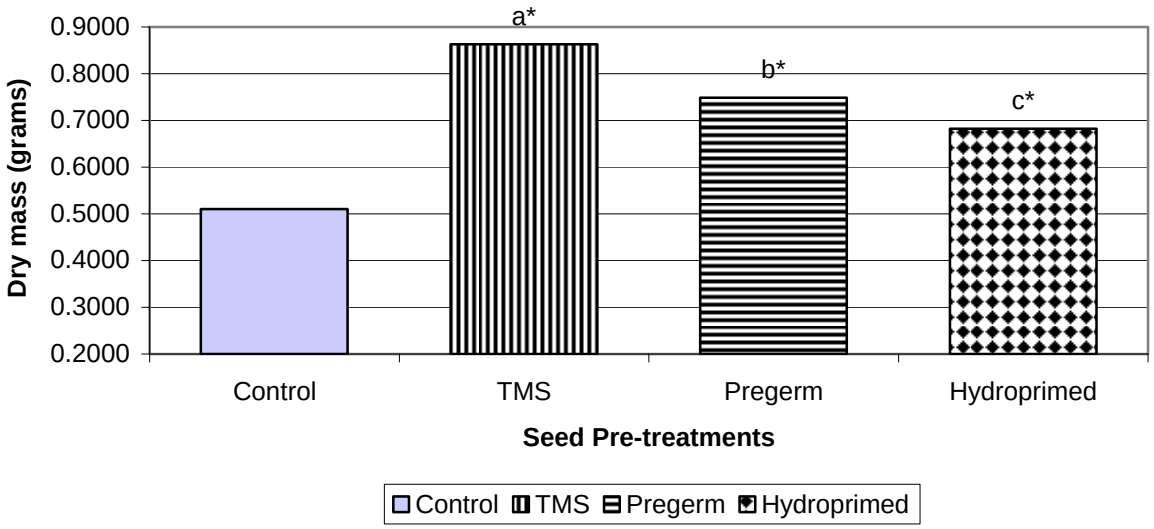

Figure 4.6. Mean dry mass of *P. taeda* seedlings in response to various seed treatments.

Treatments with similar letters are not significantly ((p < 0.001) LSD = 0.05982 grams) different to each other treatments marked with an asterisk are significantly different from the control.

From Trial 3 the following observations were made:

- The TMS treatment gave significantly ($p < 0.005$) the best growth but the poorest emergence.
- The pre-germ treatment gave the significantly ($p < 0.005$) best emergence and second best growth results (Figure 4.2).
- The hydroprimed seed gave the third best emergence and significantly ($p < 0.005$) lower growth results from the other two pre-treatments.

As has been stated earlier, obtaining best emergence is the primary aim of the seedling production system being tested. Obtaining better early growth is a secondary aim. As can be seen from Trial 3, *P. taeda* seedling emergence results, the pre-germ method of seed pre-treatment gave the greatest seedling emergence (61.13%) while the TMS method gave the worst emergence (53.38%) (LSD = 2.202%) From the results of Total Plant Growth of *P. taeda* the TMS method gave the best growth (0.8629±0.00631 g) and pre-germ

significantly less ($p < 0.005$) growth (0.7483±0.00706 g) (LSD=0.05982g). The hydroprimed method gave the lowest growth (mean: 0.6820±0.00433grams). (Figure 4.5).

The dry-sown seed which was planted into the different media type acted as a second control. These treatments were included so the effect of the different media treatments on dry-sown seed could be examined in relation to the control and the seed having received the standard seed pre-treatments. None of the dry-sown treatments were significantly ($p < 0.005$) different from the control. This demonstrates that the inclusion of smoke in the media had no noticeable effect on the emergence of *P. elliottii* of this seed lot (Figure 4.5).

Due to the fact that the treatment that gave the best emergence (pre-germ) did not give the best growth no further discussion of the effects the treatments had on plant growth is envisaged, other than to say there is no evidence to show the addition of a smoke treatment had any beneficial effect on the early plant growth of *P. taeda* (of this seed lot).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

In accordance with the research aims stated in Chapter 3 the following conclusions can be drawn and recommendations are made where applicable.

5.1 *P. elliottii* in Petri dish conditions

For *P. elliottii* sown in Petri dishes, smoke played no significant role in promoting germination. However, the method of seed pre-treatment had a significant ($p < 0.001$) effect. It is thus recommended that when testing seed in Petri dishes, the standard pre-germination method used by Sappi be applied alongside a control of dry-sown seed to assess the potential germination of *P. elliottii* seed. Although the trial tested only one seed lot, there are no indications that it may be worth re-testing different seed lots of the same species.

5.2 *P. taeda* in Petri dish conditions

For *P. taeda* sown in Petri dishes, the interaction of smoke and seed pre-treatments was significant ($p < 0.001$). The interaction of the smoke and rinse treatment in combination with the TMS seed pre-treatment was the only combination where the inclusion of smoke produced a significantly ($P < 0.005$) better result than the best pre-germination treatment method. This particular combination was the only instance in the entire study where the inclusion of smoke resulted in a significantly ($P < 0.005$) better germination of seed. The increase in seed germination by 7.19% is considered an economically worthwhile treatment in the forestry industry.

5.3 Testing under nursery conditions

When the same combination of smoke and seed pre-treatments were tested for emergence under nursery conditions no significant effect of smoke could be detected. The TMS seed pre-treatment and smoke combination that gave a significantly ($p < 0.005$) better germination than the control in the Petri dish environment was not significantly different from the control under nursery conditions. That both the pre-germination and hydropriming methods of seed pre-treatment performed better than the control under nursery conditions while not doing so in Petri dish conditions cannot be explained in the present study. These findings suggest that seed performance obtained in Petri dish environment may give very different results in a nursery environment.

5.4 Comparable results in the literature surveyed

In the literature surveyed, no reference was found comparing results in Petri dish and nursery environments. There are recorded instances of smoke having enhanced germination in Petri dishes and emergence in nursery conditions, but few studies have compared results of the same seed across different sowing environments. This finding in no way negates the studies referred to, but introduces a cautionary note to anyone hoping to use smoke in order to get greater recovery of seed. The effects of smoke need to be tested under the conditions that seed is actually raised.

5.5 Future recommended tests

Given that only in *P. taeda* did the combination of smoke and pre-treatment method give a significantly ($p < 0.005$) greater germination in response to the addition of smoke, further tests would need to be conducted on different seed lots of this species in order to establish how robust this improvement is. Given that rinsing had a positive effect it is possible that the concentration of the smoke solution was too strong. In retesting for this effect it is recommended that differing solution strengths be incorporated into the trial design. It is

possible that the age of the seed may have had an effect. In retesting it is recommended that seed-lots of different ages be used. The smoke solution may well have a marked effect on older seed that needs "waking up" The potential beneficial effect of the combination of smoke and rinse with the TMS pre-treatment method on *P. taeda* would warrant this testing. If the effect was found to be repeatable with roughly the same magnitude of benefit then it is recommended that the following additional tests be carried out.

5.5.1 Transferring the beneficial effects achieved in the Petri dish to the nursery environment via transplantation of seed

It is recommended that tests be carried out to see if seed, having received the combination of TMS, smoke and rinse, placed in a Petri dish for varying lengths of time, when transplanted into a nursery environment carries the beneficial effects with it. It is recommended that the transplant is done before the radicles begin to emerge from the seed. Seed with exposed radicles would in all probability be too fragile to survive mechanical sowing. If the beneficial effect that the treatment has is transferable in this manner the commercial nurserymen could examine the cost benefit relationship of this method of increasing seed recovery. Where a small volume of high value seed, such as obtained from a tree breeding programme, the transfer could be done by hand at virtually any stage of seedling development.

5.5.2 Transferring the beneficial effects achieved in the Petri dish to the nursery environment via environmental manipulation

One of the major differences the seed would have experienced between the Petri dish and nursery environment would have been the watering regime. In the Petri dish, only small amounts of pre-heated (to 25◦C) distilled water would have been added to the dish from time to time. Seed in the nursery was could be subjected to leaching, due to the daily watering and the porous medium seed is sown in.

It is recommended that tests be carried out to see if after sowing suitably treated seed into nursery trays, and placing those trays in a controlled environment for a while before placing the trays in the nursery, this practice may allowed the seed to benefit from the effects of the TMS and smoke and rinse combinations in the same manner the seed in the Petri dish trial did. If tests showed that this suggested method worked. Further tests could be undertaken to determine what the optimum time needed in a controlled environment would be. In a growth chamber kept at a high humidity levels, there would be no need to water the seed for the first week or so, and thus overcome the possible detrimental effects that leaching, caused by daily watering, may be having on the seed.

5.6 Keeping abreast of new developments with regard to application of smoke extracts

The results showed that the addition of smoke had no significant beneficial effect with either species under nursery conditions. A wide range of combinations of seed pre-treatments and different smoke applications were tested. It is not recommended that any further investigations be carried out on these two species at this point in time. Use of smoke as a seed germination enhancer has only recently been discovered. As further research is undertaken and new and better methods of applying smoke may be found. If future research shows that applying the butentolide compound, in it isolated or synthesized form, is preferable to that of using a smoke solution it would be worthwhile testing these products

As *P. elliottii* and *P. taeda* are widely planted in the southern United States of America, it may prove useful to review the published literature from time to time to see if any tests on these species are conducted. Some new way of applying the simulative affect of smoke may be developed. The application of smoke solution to enhance seed germination has only recently become widely known. Most of the research conducted to date has focused on understanding the mechanisms of how stimulatory properties work, and identifying what the active compound is that causes the stimulation. As more work is done in testing the application of this technology, some interesting results may come to light. The overall low germination results of the controls may indicate that the seed was not at its optimum

physiological state. However, nothing in the results obtained gives any indication that any further investigations (except the tests recommended above) should be carried out on these two species at this stage. Although no general positive results were obtained from the two species tested, Sappi could consider testing these combinations of smoke and pre-treatments on other pine species, since responses to smoke are species-dependent and many species respond positively.

5.7 Effects of smoke on plant growth in the nursery for *P. elliottii*

For *P. elliottii* there were significant ($p < 0.002$) interactions between smoke, seed pre-treatments and early plant growth. These, however, followed no distinguishable pattern. As it is concluded that the addition of smoke does not promote seedling emergence in this species in the nursery, the complex effects on growth, may be interesting, but are not worth further investigation.

5.8 Effects of smoke on plant growth in the nursery for *P. taeda*

For *P. taeda,* the TMS pre-treatment resulted in significantly better early seedling growth but it displayed the poorest emergence in the nursery. The application of smoke was not significant. As was stated earlier, the primary requirement is improved seed emergence. If the tests recommended in Sections 5.1 and 5.2 capture the beneficial results of the smoke and TMS interaction, the significantly improved early seedling growth would be an added benefit.

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APPENDICES

APPENDIX 1

ANOVA – P. elliotti Trial 2 Transformed Data

253 Analysis of variance

Variate: ArcSineTotal

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Control	1	220.71	220.71	2.7	0.101
Control.Method	2	19109.99	9555	117.09	<.001
Control.Smoke	2	129.44	64.72	0.79	0.454
Control.Method.Smoke	4	555.24	138.81	1.7	0.15
Residual	240	19585.38	81.61		
Total	249	39600.76			

Message: the following units have large residuals.

units 47	-25.64	approx. s.e.	8.85
units 194	26.56	approx. s.e.	8.85
units 230	-27.24	approx. s.e.	8.85
units 233	-27.24	approx. s.e.	8.85
units 237	25.89	approx. s.e.	8.85

Tables of means

Variate: ArcSineTotal

Grand mean 24.42

Control	0	1
	24.11	27.24
rep.	225	25

Control	Method	Drysown	Hydro	Pregerm	TMS
0			**25.45**	**34.66**	**12.21**
	rep.		75	75	75
1		27.24			

		rep.	25			
Control	Smoke	control	Smoke	Smoke+rinse	Water	
0			25.07	23.22	24.03	
	rep.		75	75	75	
1		27.24				
	rep.	25				

Control	Method	Smoke	control	Smoke	Smoke+rinse	Water
0	Hydro			25.64	23.23	27.49
	Pregerm			34.59	33.96	35.44
	TMS			15	12.47	9.16
1	Drysown		27.24			

Standard errors of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
e.s.e.	1.807	1.807	1.807	1.807	min.rep
	0.602	1.043	1.043		max.rep

Standard errors of differences of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
s.e.d.		2.555X	2.555X		min.rep
	1.904	2.086	2.086	2.555	max-min
		1.475	1.475		max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Control	Control	Control	Control	

		Method	Smoke	Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
l.s.d.		5.033X	5.033X		min.rep
	3.752	4.11	4.11	5.033	max-min
		2.906	2.906		max.rep

(No comparisons in categories where l.s.d. marked with an X)

Stratum standard errors and coefficients of variation

Variate: ArcSineTotal

d.f.	s.e.	cv%
240	9.034	37

APPENDIX 2

ANOVA - P. taeda Germination
Trial 2

245 Analysis of variance

Variate: ArcSineTotal

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Control	1	137.09	137.09	1.97	0.162
Control.Method	2	6953.41	3476.71	49.95	<.001
Control.Smoke	2	612.96	306.48	4.4	0.013
Control.Method.Smoke	4	1452.47	363.12	5.22	<.001
Residual	240	16705.94	69.61		
Total	249	25861.86			

Message: the following units have large residuals.

units 60	28.02	approx. s.e.	8.17
units 84	32.7	approx. s.e.	8.17
units 146	26.68	approx. s.e.	8.17

Tables of means

Variate: ArcSineTotal

Grand mean 54.85

Control	0	1
	55.1	52.63
rep.	225	25

Control	Method	Drysown	Hydro	Pregerm	TMS
0			48.86	54.08	62.36
	rep.		75	75	75
1		52.63			
	rep.	25			

Control	Smoke	control	Smoke	Smoke+rinse	Water
0			52.94	56.95	55.41

		rep.		75	75	75	
	1		52.63				
		rep.	25				
	Control	Method	Smoke	control	Smoke	Smoke+rinse	Water
	0	Hydro			46.8	50.44	49.33
		Pregerm			53.16	51.78	57.3
		TMS			**58.87**	**68.62**	**59.58**
	1	Drysown		52.63			

Standard errors of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
e.s.e.	1.669	1.669	1.669	1.669	min.rep
	0.556	0.963	0.963		max.rep

Standard errors of differences of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
s.e.d.		2.360X	2.360X		min.rep
	1.759	1.927	1.927	2.36	max-min
		1.362	1.362		max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
l.s.d.		4.649X	4.649X		min.rep
	3.465	3.796	3.796	4.649	max-min

	2.684	2.684	max.rep

(No comparisons in categories where l.s.d.
marked with an X)

Stratum standard errors and
coefficients of variation

Variate: ArcSineTotal

d.f.	s.e.	cv%
240	8.343	15.2

APPENDIX 3

ANOVA - P. taeda Emergance Trial 3

245 Analysis of variance

Variate: ArcSineTotal

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Control	1	137.09	137.09	1.97	0.162
Control.Method	2	6953.41	3476.71	49.95	<.001
Control.Smoke	2	612.96	306.48	4.4	0.013
Control.Method.Smoke	4	1452.47	363.12	5.22	<.001
Residual	240	16705.94	69.61		
Total	249	25861.86			

Message: the following units have large residuals.

units 60	28.02	approx. s.e.	8.17
units 84	32.7	approx. s.e.	8.17
units 146	26.68	approx. s.e.	8.17

Tables of means

Variate: ArcSineTotal

Grand mean 54.85

Control	0	1
	55.1	52.63
rep.	225	25

Control	Method	Drysown	Hydro	Pregerm	TMS
0			48.86	54.08	62.36
	rep.		75	75	75
1		52.63			
	rep.	25			

Control	Smoke	control	Smoke	Smoke+rinse	Water
0			52.94	56.95	55.41
	rep.		75	75	75
1		52.63			
	rep.	25			

Control	Method	Smoke	control	Smoke	Smoke+rinse	Water
0	Hydro			46.8	50.44	49.33
	Pregerm			53.16	51.78	57.3
	TMS			**58.87**	**68.62**	**59.58**
1	Drysown		52.63			

Standard errors of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
e.s.e.	1.669	1.669	1.669	1.669	min.rep
	0.556	0.963	0.963		max.rep

Standard errors of differences of means

Table	Control	Control Method	Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
s.e.d.		2.360X	2.360X		min.rep
	1.759	1.927	1.927	2.36	max-min
		1.362	1.362		max.rep

(No comparisons in categories where s.e.d. marked with an X)

Least significant differences of means (5% level)

Table	Control	Control Method	Control Smoke	Control Method

				Smoke	
rep.	unequal	unequal	unequal	25	
d.f.	240	240	240	240	
l.s.d.		4.649X	4.649X		min.rep
	3.465	3.796	3.796	4.649	max-min
		2.684	2.684		max.rep

(No comparisons in categories where l.s.d. marked with an X)

Stratum standard errors and coefficients of variation

Variate: ArcSineTotal

d.f.	s.e.	cv%
240	8.343	15.2

APPENDIX 4

Analysis of variance Trial 3 *P.elliottii*
Emergence Transformed Data

Variate: AngPerEmerg

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	11		510.94	46.45	1.48	
Rep.Plant_medium stratum						
Plant_medium	2		527.44	263.72	8.4	0.002
Residual	22		690.68	31.39	0.94	
Rep.Plant_medium.Treat stratum						
Control	1		880.2	880.2	26.44	<.001
Plant_medium.Control	2		561.64	280.82	8.43	<.001
Control.Method	2		7941.22	3970.61	119.26	<.001
Control.Smoke	2		134.5	67.25	2.02	0.134
Plant_medium.Control.Method						
Plant_medium.Control.Smoke	4		672.55	168.14	5.05	<.001
Control.Method.Smoke	4		416.94	104.24	3.13	0.015
Plant_medium.Control.Method.Smoke	4		509.36	127.34	3.82	0.005
	8		538.08	67.26	2.02	0.044
Residual	296	-1	9855.06	33.29		
Total	358	-1	23183.72			

Message: the following units have large residuals.

Rep 12	-2.71	approx. s.e. 1.19

Rep 11 Plant_medium Bark+smoke+leach	-2.83	approx. s.e. 1.39
Rep 1 Plant_medium Bark+smoke+leach Treat 5.	-20.48	approx. s.e. 5.23
Rep 3 Plant_medium Bark+smoke+leach Treat 5.	-18.88	approx. s.e. 5.23
Rep 6 Plant_medium Bark+smoke+leach Treat 6.	23.89	approx. s.e. 5.23
Rep 7 Plant_medium Bark Treat 5.	-16.2	approx. s.e. 5.23

Tables of means

Variate: AngPerEmerg

Grand mean 30.40

Plant_medium	Bark+smoke	Bark	Bark+smoke+leach
	28.97	31.93	30.3

Control	1	2
	30.92	25.71
rep.	324	36

Plant_medium	Control	1	2
Bark+smoke		28.91	29.52
	rep.	108	12
Bark		32.82	23.96
	rep.	108	12
Bark+smoke+leach		31.04	23.65
	rep.	108	12

Control	Method	Hydro	TMS	Pregerm	Drysown
1		34.37	23.92	34.47	
	rep.	108	108	108	
2					25.71
	rep.				36

Control	Smoke	water	smoke	smoke+rinsed	control
1		31.81	30.31	30.64	
	rep.	108	108	108	
2					25.71
	rep.				36

Plant_medium	Control	Method	Hydro	TMS	Pre-germ	Dry-sown
Bark+smoke	1		30.76	24.63	31.33	
		rep.	36	36	36	
	2					29.52
		rep.				12
Bark	1		36.33	25.02	37.1	
		rep.	36	36	36	
	2					23.96
		rep.				12
Bark+smoke+leach	1		36.03	22.11	34.97	
		rep.	36	36	36	
	2					23.65
		rep.				12

Plant_medium	Control	Smoke	water	smoke	smoke+rinsed
Bark+smoke	1		28.95	26.95	30.83
		rep.	36	36	36
Bark	1		34.01	33.3	31.14
		rep.	36	36	36
Bark+smoke+leach	1		32.47	30.68	29.96
		rep.	36	36	36

Plant_medium	Control	Smoke	control
Bark+smoke	2		29.52
		rep.	12
Bark	2		23.96
		rep.	12
Bark+smoke+leach	2		23.65
		rep.	12

Control	Method	Smoke	water	smoke	smoke+rinsed	Control
1	Hydro		33.64	34.23	35.26	
	TMS		25.88	24.33	21.55	
	Pre-germ		35.92	32.37	35.12	
2	Dry-sown					25.71

Plant medium	Control	Method	Smoke	water	smoke
Bark+smoke	1	Hydro		27.97	28.51
		TMS		27.59	24.11
		Pregerm		31.3	28.23
Bark	1	Hydro		35.8	36.91
		TMS		26.73	27.94
		Pregerm		39.5	35.05
Bark+smoke+leach	1	Hydro		37.14	37.26
		TMS		23.32	20.94
		Pregerm		36.95	33.82

Plant_medium	Control	Method	Smoke	smoke+rinsed	control
Bark+smoke	1	Hydro		35.8	
		TMS		22.2	
		Pregerm		34.47	
	2	Drysown			29.52
Bark	1	Hydro		36.28	
		TMS		20.39	
		Pregerm		36.75	
	2	Drysown			23.96
Bark+smoke+leach	1	Hydro		33.69	
		TMS		22.07	
		Pregerm		34.14	
	2	Drysown			23.65

Standard errors of differences of means

Table	Plant_medium	Control	Plant_medium Control	Control Method
rep.	120	unequal	unequal	unequal

s.e.d.			2.349	1.360X	min.rep
d.f.			314.78	296	
					max-
s.e.d.	0.723	1.014	1.747	1.11	min
d.f.	22	296	273.54	296	
s.e.d.			0.765	0.785	max.rep
d.f.			27.46	296	

Except when comparing means with the same level(s) of

Plant_medium			2.356		min.rep
d.f.			296		
					max-
			1.756		min
d.f.			296		
			0.785		max.rep
d.f.			296		

Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
s.e.d.	1.360X	2.349	2.349		min.rep
d.f.	296	314.78	314.78		
					max-
s.e.d.	1.11	1.915	1.915	1.36	min
d.f.	296	293.4	293.4	296	
s.e.d.	0.785	1.348	1.348		max.rep
d.f.	296	182.52	182.52		

Except when comparing means with the same level(s) of

Plant_medium		2.356	2.356		min.rep
d.f.		296	296		
					max-
		1.923	1.923		min
d.f.		296	296		
		1.36	1.36		max.rep
d.f.		296	296		

Plant_medium.Control

		2.356	2.356		min.rep
d.f.		296	296		
					max-
		1.923	1.923		min
d.f.		296	296		
		1.36	1.36		max.rep

d.f.		296	296
Table	Plant_medium		
	Control		
	Method		
	Smoke		
rep.	12		
s.e.d.	2.349		
d.f.	314.78		

Except when comparing means with the same level(s) of

Plant_medium	2.356
d.f.	296
Plant_medium.Control	2.356
d.f.	296
Plant_medium.Control.Method	2.356
d.f.	296
Plant_medium.Control.Smoke	2.356
d.f.	296

(No comparisons in categories where s.e.d. marked with an X)
(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
l.s.d.			4.622	2.677X	min.rep
d.f.			314.78	296	
l.s.d.	1.5	1.995	3.439	2.185	max-min
d.f.	22	296	273.54	296	
l.s.d.			1.568	1.545	max.rep

d.f.			27.46	296	
Except when comparing means with the same level(s) of					
Plant_medium			4.636		min.rep
d.f.			296		
			3.455		max-min
d.f.			296		
			1.545		max.rep
d.f.			296		
Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
l.s.d.	2.677X	4.622	4.622		min.rep
d.f.	296	314.78	314.78		
l.s.d.	2.185	3.769	3.769	2.677	max-min
d.f.	296	293.4	293.4	296	
l.s.d.	1.545	2.66	2.66		max.rep
d.f.	296	182.52	182.52		
Except when comparing means with the same level(s) of					
Plant_medium		4.636	4.636		min.rep
d.f.		296	296		
		3.785	3.785		max-min
d.f.		296	296		
		2.677	2.677		max.rep
d.f.		296	296		
Plant_medium.Control					
		4.636	4.636		min.rep
d.f.		296	296		
		3.785	3.785		max-min
d.f.		296	296		
		2.677	2.677		max.rep
d.f.		296	296		
Table	Plant_medium Control Method Smoke				

rep.	12
l.s.d.	4.622
	314.78
d.f. Except when comparing means with the same level(s) of Plant_medium	4.636
d.f. Plant_medium.Control	296
	4.636
d.f. Plant_medium.Control. Method	296
	4.636
d.f. Plant_medium.Control. Smoke	296
	4.636
d.f.	296

(No comparisons in categories where l.s.d. marked with an X)

(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

Variate: AngPerEmerg

Stratum	d.f.	s.e.	cv%
Rep	11	1.244	4.1
Rep.Plant_medium	22	1.772	5.8
Rep.Plant_medium.Tre	296	5.77	19

at

1041 APLOT [RMETHOD=simple] fitted,normal,halfnormal,histogram

1042 AGRAPH [METHOD=lines] Plant_medium; Control

APPENDIX 5

Analysis of variance Trial 3 *P. elliottii*
Total Plant Growth

Variate:
TotalPlant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	11		0.42708	0.03883	1.31	
Rep.Plant_medium stratum						
Plant_medium	2		0.94448	0.47224	15.96	<.001
Residual	22		0.65081	0.02958	0.9	
Rep.Plant_medium.Treat stratum						
Control	1		1.77572	1.77572	53.89	<.001
Plant_medium.Control	2		0.23209	0.11604	3.52	0.031
Control.Method	2		6.43656	3.21828	97.66	<.001
Control.Smoke	2		0.13088	0.06544	1.99	0.139
Plant_medium.Control.Method	4		0.48496	0.12124	3.68	0.006
Plant_medium.Control.Smoke						
Control.Method.Smoke	4		0.45571	0.11393	3.46	0.009
Plant_medium.Control.Method.Smoke	4		0.56897	0.14224	4.32	0.002
	8		0.58605	0.07326	2.22	0.026
Residual	294	-3	9.68803	0.03295		
Total	356	-3	22.22749			

Message: the following units have large residuals.

Rep 10		approx.
Plant_medium		s.e.
Bark+smoke	-0.0917	0.0425
Rep 11		approx.
Plant_medium		s.e.
Bark	0.0899	0.0425
Rep 11		
Plant_medium		approx.
Bark+smoke+leach	-0.0971	s.e. 0.0425

Rep 1		
Plant_medium		approx.
Bark+smoke+leach Treat 10.	0.7615	s.e. 0.1640
Rep 4		
Plant_medium		approx.
Bark+smoke+leach Treat 6.	0.5288	s.e. 0.1640

Tables of means

Variate: TotalPlant

Grand mean 0.5552

Plant_medium	Bark+smoke	Bark	Bark+smoke+leach
	0.4848	0.6053	0.5753

Control	1	2	
	0.5786	0.3445	
rep.	324	36	

Plant_medium			
Bark+smoke	Control	1	2
		0.4976	0.3699
	rep.	108	12

Bark		0.6388	0.3041			
	rep.	108	12			
Bark+smoke+leach		0.5993	0.3594			
	rep.	108	12			

Control	Method	Hydro	TMS	Pregerm	Drysown	
1		0.6811	0.3793	0.6753		
	rep.	108	108	108		
2					0.3445	
	rep.				36	

Control	Smoke	water	smoke	smoke+rinsed	control	
1		0.6068	0.5672	0.5617		
	rep.	108	108	108		
2					0.3445	
	rep.				36	

Plant_medium	Control	Method	Hydro	TMS	Pregerm	Drysown
Bark+smoke	1		0.5656	0.3689	0.5583	
		rep.	36	36	36	
	2					0.3699
		rep.				12
Bark	1		0.7315	0.4142	0.7708	
		rep.	36	36	36	
	2					0.3041
		rep.				12
Bark+smoke+leach	1		0.7463	0.3548	0.6968	
		rep.	36	36	36	
	2					0.3594
		rep.				12

Plant_medium	Control	Smoke	water	smoke	smoke+rinsed
Bark+smoke	1		0.4967	0.4476	0.5485
		rep.	36	36	36
Bark	1		0.664	0.6738	0.5787
		rep.	36	36	36

Bark+smoke+leach	1	rep.	0.659736	0.580136	0.55836	
Plant_medium	Control	Smoke	control			
Bark+smoke	2	rep.	0.369912			
Bark	2	rep.	0.304112			
Bark+smoke+leach	2	rep.	0.359412			

Control	Method	Smoke	water	smoke	smoke+rinsed	control	
1	Hydro		0.6547	0.6638	0.7249		
	TMS		0.4289	0.4114	0.2976		
	Pregerm		0.7369	0.6263	0.6627		
2	Drysown					0.3445	

Plant_medium	Control	Method	Smoke	water	smoke	smoke+rinsed	Control
Bark+smoke	1	Hydro		0.4839	0.4794	0.7336	
		TMS		0.4397	0.3933	0.2735	
		Pregerm		0.5666	0.4699	0.6384	
	2	Drysown					0.3699
Bark	1	Hydro		0.6969	0.7783	0.7192	
		TMS		0.4638	0.517	0.2617	
		Pregerm		0.8312	0.7261	0.7552	
	2	Drysown					0.3041
Bark+smoke+leach	1	Hydro		0.7832	0.7337	0.722	
		TMS		0.383	0.3237	0.3576	
		Pregerm		0.8131	0.6829	0.5944	
	2	Drysown					0.3594

Standard errors of means

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
e.s.e.	0.0157	0.03025	0.05213	0.03025	min.rep
d.f.	22	294	313.85	294	
e.s.e.		0.01008	0.01664	0.01747	max.rep
d.f.		294	27.75	294	
Except when comparing means with the same level(s) of Plant_medium					
			0.0524		min.rep
d.f.			294		
			0.01747		max.rep
d.f.			294		

Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
e.s.e.	0.03025	0.05213	0.05213	0.03025	min.rep
d.f.	294	313.85	313.85	294	
e.s.e.	0.01747	0.02979	0.02979		max.rep
d.f.	294	189.29	189.29		
Except when comparing means with the same level(s) of Plant_medium					
		0.0524	0.0524		min.rep
d.f.		294	294		
		0.03025	0.03025		max.rep
d.f.		294	294		
Plant_medium. Control					
		0.0524	0.0524		min.rep
d.f.		294	294		
		0.03025	0.03025		max.rep
d.f.		294	294		

Table	Plant_medium Control Method Smoke

rep.	12
e.s.e.	0.05213
d.f.	313.85
Except when comparing means with the same level(s) of	
Plant_medium	0.0524
d.f.	294
Plant_medium. Control	
	0.0524
d.f.	294
Plant_medium. Control.Method	
	0.0524
d.f.	294
Plant_medium. Control.Smoke	
	0.0524
d.f.	294

(Not adjusted for missing values)

Standard errors of differences of means

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
s.e.d.			0.07373	0.04279 X	min.rep
d.f.			313.85	294	
s.e.d.	0.0222	0.03189	0.05473	0.03494	max-min
d.f.	22	294	277.36	294	
s.e.d.			0.02354	0.0247	max.rep
d.f.			27.75	294	
Except when comparing means with the same level(s) of					
Plant_medium			0.07411		min.rep
d.f.			294		

					max-min
			0.05524		
d.f.			294		
			0.0247		max.rep
d.f.			294		
Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
s.e.d.	0.04279 X	0.07373	0.07373		min.rep
d.f.	294	313.85	313.85		
					max-min
s.e.d.	0.03494	0.06004	0.06004	0.04279	
d.f.	294	295.44	295.44	294	
s.e.d.	0.0247	0.04213	0.04213		max.rep
d.f.	294	189.29	189.29		
Except when comparing means with the same level(s) of					
Plant_medium		0.07411	0.07411		min.rep
d.f.		294	294		
					max-min
		0.06051	0.06051		
d.f.		294	294		
		0.04279	0.04279		max.rep
d.f.		294	294		
Plant_medium. Control					
		0.07411	0.07411		min.rep
d.f.		294	294		
					max-min
		0.06051	0.06051		
d.f.		294	294		
		0.04279	0.04279		max.rep
d.f.		294	294		
Table	Plant_medium Control Method Smoke				
rep.	12				
s.e.d.	0.07373				
d.f.	313.85				

Except when comparing means with the same level(s) of Plant_medium	0.07411				
d.f.	294				
Plant_medium. Control		0.07411			
d.f.		294			
Plant_medium. Control.Method			0.07411		
d.f.			294		
Plant_medium. Control.Smoke				0.07411	
d.f.				294	

(No comparisons in categories where s.e.d. marked with an X)
(Not adjusted for missing values)

Least significant differences of means (5% level)

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
l.s.d.			0.14506	0.08421 X	min.rep
d.f.			313.85	294	
l.s.d.	0.04605	0.06276	0.10773	0.06875	max-min
d.f.	22	294	277.36	294	
l.s.d.			0.04824	0.04862	max.rep
d.f.			27.75	294	
Except when comparing means with the same level(s) of Plant_medium			0.14585		min.rep
d.f.			294		

					max-
			0.10871		min
d.f.			294		
			0.04862		max.rep
d.f.			294		
Table	Control Smoke	Plant_medium Control Method Smoke	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
l.s.d.	0.08421 X	0.14506	0.14506		min.rep
d.f.	294	313.85	313.85		
					max-
l.s.d.	0.06875	0.11817	0.11817	0.08421	min
d.f.	294	295.44	295.44	294	
l.s.d.	0.04862	0.08309	0.08309		max.rep
d.f.	294	189.29	189.29		
Except when comparing means with the same level(s) of Plant_medium		0.14585	0.14585		min.rep
d.f.		294	294		
					max-
		0.11909	0.11909		min
d.f.		294	294		
		0.08421	0.08421		max.rep
d.f.		294	294		
Plant_medium. Control					
		0.14585	0.14585		min.rep
d.f.		294	294		
					max-
		0.11909	0.11909		min
d.f.		294	294		
		0.08421	0.08421		max.rep
d.f.		294	294		
Table	Plant_medium Control Method Smoke				
rep.	12				
l.s.d.	0.14506				
d.f.	313.85				

Except when comparing means with the same level(s) of Plant_medium	0.14585
d.f.	294
Plant_medium.Control	0.14585
d.f.	294
Plant_medium.Control.Method	0.14585
d.f.	294
Plant_medium.Control.Smoke	0.14585
d.f.	294

(No comparisons in categories where l.s.d. marked with an X)
(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

Variate: TotalPlant

Stratum	d.f.	s.e.	cv%
Rep	11	0.03597	6.5
Rep.Plant_medium	22	0.05439	9.8
Rep.Plant_medium.Treat	294	0.18153	32.7

APPENDIX 6

Analysis of variance Trial 3 *P.taeda*
TOTAL PLANT GROWTH

Variate:
TotalPlant

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	11		1.96335	0.17849	8.14	
Rep.Plant_medium stratum						
Plant_medium	2		0.19377	0.09688	4.42	0.024
Residual	22		0.48269	0.02194	0.88	
Rep.Plant_medium.Treat stratum						
Control	1		2.09538	2.09538	83.99	<.001
Plant_medium.Control	2		0.04678	0.02339	0.94	0.393
Control.Method	2		1.8083	0.90415	36.24	<.001
Control.Smoke	2		0.0181	0.00905	0.36	0.696
Plant_medium.Control.Method	4		0.16692	0.04173	1.67	0.156
Plant_medium.Control.Smoke						
Control.Method.Smoke	4		0.0719	0.01797	0.72	0.578
Plant_medium.Control.Method.Smoke	4		0.04741	0.01185	0.48	0.754
	8		0.19421	0.02428	0.97	0.457
Residual	296	-1	7.38433	0.02495		
Total	358	-1	14.4718			

Message: the following units have large residuals.

		approx. s.e.
Rep 1	-0.1497	0.0738
		approx. s.e.
Rep 11	0.1485	0.0738

Rep 10 Plant_medium		approx. s.e.
Bark+smoke	0.1283	0.0366
Rep 10 Plant_medium		approx. s.e.
Bark	-0.0946	0.0366

Rep 2 Plant_medium		approx. s.e.
Bark Treat 6.	0.6519	0.1432
Rep 7 Plant_medium Bark+smoke		approx. s.e.
Treat 5.	-0.4552	0.1432
Rep 9 Plant_medium Bark+smoke+le		approx. s.e.
ach Treat 7.	-0.7117	0.1432
Rep 12 Plant_medium Bark+smoke+le		approx. s.e.
ach Treat 9.	-0.5327	0.1432

Tables of means

Variate: TotalPlant	Plant_medium	Bark+smoke	Bark	Bark+smoke+leach
		0.7659	0.7418	0.7093
Grand mean 0.7390				
	Control	1	2	
		0.7644	0.5101	
	rep.	324	36	

Plant_medium	Control	1	2
Bark+smoke		0.7867	0.578
	rep.	108	12
Bark		0.772	0.4703
	rep.	108	12
Bark+smoke+leach		0.7345	0.4821
	rep.	108	12
Bark		0.772	0.4703
	rep.	108	12
Bark+smoke+leach		0.7345	0.4821
	rep.	108	12

Control	Method	Hydro	TMS	Pregerm	Dryown
1		0.682	0.8629	0.7483	
	rep.	108	108	108	
2					0.510136
	rep.				

Control	Smoke	water	smoke	smoke+rinsed	control
1		0.7686	0.7708	0.7539	
	rep.	108	108	108	
2					0.510136
	rep.				

Plant_medium	Control	Method	Hydro	TMS	Pregerm	Dryown
Bark+smoke	1		0.7295	0.8404	0.7903	
		rep.	36	36	36	
	2					0.578
		rep.				12
Bark	1		0.6768	0.8981	0.7412	
		rep.	36	36	36	
	2					0.4703
		rep.				12
Bark+smoke+leach	1		0.6399	0.8502	0.7135	
		rep.	36	36	36	
	2					0.4821

		rep.				12

Plant_medium	Control	Smoke	water	smoke	smoke +rinsed
Bark+smoke	1		0.767436	0.809336	0.783636
		rep.			
Bark	1		0.793536	0.755236	0.767436
		rep.			
Bark+smoke+leach	1		0.744836	0.747936	0.710936
		rep.			

Plant_medium	Control	Smoke	control
Bark+smoke	2		0.578
		rep.	12
Bark	2		0.4703
		rep.	12
Bark+smoke+leach	2		0.4821
		rep.	12

	Control	Method	Smoke	water	smoke	smoke +rinsed	Control
	1	Hydro		0.7053	0.6755	0.6653	
		TMS		0.8461	0.8856	0.8571	
		Pregerm		0.7543	0.7513	0.7394	
	2	Drysown					0.510

Plant_medium	Control	Method	Smoke	water	smoke	smoke +rinsed	Control
Bark+smoke	1	Hydro		0.7667	0.7557	0.6661	
		TMS		0.7967	0.8518	0.8729	
		Pregerm		0.7389	0.8203	0.8117	
	2	Drysown					0.578
Bark	1	Hydro		0.6843	0.6655	0.6806	
		TMS		0.9145	0.8867	0.8931	
		Pregerm		0.7818	0.7134	0.7284	
	2	Drysown					0.470

	Bark+smoke+leach	1	Hydro	0.665	0.6053	0.6493	
			TMS	0.827	0.9183	0.8053	
			Pregerm	0.7423	0.7202	0.678	
		2	Drysown				0.482

Standard errors of differences of means

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
s.e.d.			0.06409	0.03723X	min.rep
d.f.			316.07	296	
					max-min
s.e.d.	0.01912	0.02775	0.04754	0.0304	
d.f.	22	296	280.61	296	
s.e.d.			0.02029	0.02149	max.rep
d.f.			27.88	296	
Except when comparing means with the same level(s) of Plant_medium			0.06448		min.rep
d.f.			296		
			0.04806		max-min
d.f.			296		
			0.02149		max.rep
d.f.			296		

Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
s.e.d.	0.03723X	0.06409	0.06409		min.rep
d.f.	296	316.07	316.07		
					max-min
s.e.d.	0.0304	0.05217	0.05217	0.03723	
d.f.	296	298.33	298.33	296	
s.e.d.	0.02149	0.03655	0.03655		max.rep
d.f.	296	192.75	192.75		
Except when comparing means with the same level(s) of Plant_medium		0.06448	0.06448		min.rep
d.f.		296	296		
		0.05265	0.05265		max-

			min
d.f.	296	296	
	0.03723	0.03723	max.rep
d.f.	296	296	
Plant_medium. Control			
	0.06448	0.06448	min.rep
d.f.	296	296	
			max-min
	0.05265	0.05265	
d.f.	296	296	
	0.03723	0.03723	max.rep
d.f.	296	296	

Table	Plant_medium Control Method Smoke
rep.	12
s.e.d.	0.06409
d.f.	316.07

Except when comparing means with the same level(s) of

Plant_medium	0.06448
d.f.	296
Plant_medium. Control	
	0.06448
d.f.	296
Plant_medium. Control.Method	
	0.06448
d.f.	296
Plant_medium. Control.Smoke	
	0.06448
d.f.	296

(No comparisons in categories where s.e.d. marked with an X)
(Not adjusted for missing values)

Least significant differences
of means (5% level)

Table	Plant_medium	Control	Plant_medium Control	Control Method	
rep.	120	unequal	unequal	unequal	
l.s.d.			0.1261	0.07327X	min.rep
d.f.			316.07	296	
l.s.d.	0.03966	0.05461	0.09358	0.05982	max-min
d.f.	22	296	280.61	296	
l.s.d.			0.04158	0.0423	max.rep
d.f.			27.88	296	
Except when comparing means with the same level(s) of Plant_medium			0.1269		min.rep
d.f.			296		
			0.09459		max-min
d.f.			296		
			0.0423		max.rep
d.f.			296		

Table	Control Smoke	Plant_medium Control Method	Plant_medium Control Smoke	Control Method Smoke	
rep.	unequal	unequal	unequal	36	
l.s.d.	0.07327X	0.1261	0.1261		min.rep
d.f.	296	316.07	316.07		
l.s.d.	0.05982	0.10267	0.10267	0.07327	max-min
d.f.	296	298.33	298.33	296	
l.s.d.	0.0423	0.07209	0.07209		max.rep
d.f.	296	192.75	192.75		
Except when comparing means with the same level(s) of Plant_medium		0.1269	0.1269		min.rep
d.f.		296	296		
		0.10361	0.10361		max-min
d.f.		296	296		
		0.07327	0.07327		max.rep

d.f.	296	296	
Plant_medium.Control			
	0.1269	0.1269	min.rep
d.f.	296	296	
			max-min
	0.10361	0.10361	
d.f.	296	296	
	0.07327	0.07327	max.rep
d.f.	296	296	

Table	Plant_medium Control Method Smoke
rep.	12
l.s.d.	0.1261
d.f.	316.07

Except when comparing means with the same level(s) of

Plant_medium	0.1269
d.f.	296
Plant_medium.Control	0.1269
d.f.	296
Plant_medium.Control.Method	0.1269
d.f.	296
Plant_medium.Control.Smoke	0.1269
d.f.	296

(No comparisons in categories where l.s.d. marked with an X)
(Not adjusted for missing values)

Stratum standard errors and coefficients of variation

Variate: TotalPlant

Stratum	d.f.	s.e.	cv%
Rep	11	0.07713	10.4
Rep.Plant_medium	22	0.04684	6.3
Rep.Plant_medium.Treat	296	0.15795	21.4