The physiology of *Pinus patula* seedlings in response to water stress and the implications for plantation regeneration in South Africa

by

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Abstract

Pinus patula Schiede ex Schlect. & Cham. is the most widely planted softwood species for both pulpwood and saw timber in the South African forestry industry. High mortality of this species, often in excess of 20%, following planting is currently of major concern and has the potential to limit future deployment for commercial timber. Water stress is often reported to be a cause of mortality during regeneration in commercial forestry plantations yet, prior to 2007, there was no published research on the water relations of *P. patula* during regeneration in South Africa. This, together with questions raised by the industry as to the role of using water in the planting operation, initiated the series of studies conducted for this thesis. Water planting (application of water into the planting hole at the time of planting) of *P. patula* seedlings has been used commercially to reduce post-planting water stress and buffer against potentially extreme weather conditions immediately after planting. However, the primary role of the water, as well as its success in increasing survival following planting, has never been critically assessed. Since the use of water in the planting operation is expensive, it was essential that the benefits to using water were quantified, in terms of survival and growth, and justified, in terms of any monetary investment. In addition, there was a lack of local studies investigating the physiological characteristics of *P. patula* seedlings, particularly their tolerance to low soil water availability. To understand the role of water during the regeneration of *P. patula* in terms of plantation management and seedling physiology, a variety of research methodologies were used that included: applied field trials, multivariate methods (a retrospective investigation), pot trials and the development of a simple financial model.

Four field trials were implemented to test the response in *P. patula* survival to water applied at planting. Two trials each were situated in the KwaZulu-Natal (KZN) Midlands and Mpumalanga Escarpment. The first trial at each site was planted in spring (October) and the second in summer (February). Watering treatments consisted of different quantities of water used in the planting operation and included 0.5 litres, 2 litres, 4 litres and no water (dry plant). Only at the spring planted trial in the KZN Midlands was survival of the dry planted seedlings significantly lower than that of the seedlings planted with water, at 90 days after planting. This may have been due to low rainfall during the week before and two weeks after planting, or the small size of the seedlings used in the trial. Application of 0.5 litres of water to the planting pit was sufficient to increase survival to a level equivalent to

that where 2 or 4 litres of water was used, yet only increased soil moisture in the area immediately surrounding the seedling. This suggested that the role of the water applied during planting was increased root to soil contact. Overall, these four trials indicated that planting with water had the potential to increase survival only when soil water availability was low and rainfall sporadic. There was no effect of water applied at planting on early tree growth.

While the results of the four field trials provided an indication of the effect of planting with water on subsequent survival of *P. patula* seedlings, there was concern that the results of the four trials may not be a true reflection of a dynamic situation. Survival in response to water applied at planting may vary from year to year and across forestry regions due to the unpredictable nature of rainfall and high air temperatures during the planting season, as well as the wide range of forestry sites across which *P. patula* seedlings are planted. To improve our understanding, a database of 58 trials was compiled where water and dry planting had been carried out. In this way it was possible to investigate whether the results from the four field trials were reflected in a range of previously conducted field trials implemented across time and space. The trials incorporated into the dataset were all planted to *P. patula* between 1990 and 2005 in the summer rainfall region of southern Africa. Data related to the climate, local weather, physiography and site management at each trial were also included. Summary statistics, linear correlation and multiple regression were used to determine if site-associated variables were related to an increase in survival in the water relative to the dry planted treatments. The analyses indicated that for all 58 trials, survival was lowest during the summer months, regardless of planting treatment. Planting with water was most likely to increase survival when used during spring, autumn and winter planting, although (as with the four applied field trials) there was no overall significant relationship between water planting and survival.

Based on these results it was anticipated that an understanding of the water stress physiology of *P. patula* seedlings was required to explain the observed trends from a more fundamental perspective; if planting with water did not always increase survival, why not? Three pot trials were conducted to increase the understanding of the water relations of *P. patula* seedlings. These trials were also used to provide benchmark physiological data related to stressed (water) and unstressed seedlings. The first pot trial highlighted the importance of root plug moisture at the time of planting for increasing subsequent survival.

The subsequent two pot trials were aimed at investigating the interaction between planting stock quality (as determined by measures of size) and soil water availability and the effect on survival, growth and physiology of *P. patula* seedlings. These results indicated that *P. patula* seedlings were not as sensitive to high air and soil temperatures (above 30˚C) and low soil water availability (below -1.5 MPa) as previously thought. The seedlings were able to tolerate low soil water availability for several weeks and, following rewatering, were able to recover from moderate and severe water stress (a shoot water potential of below -1.5 MPa). This data supported the results from the four applied field trials and retrospective study of 58 trials, where the application of water to the seedlings at planting did not substantially increase survival. In the pot trials, stomatal conductance started to decrease when shoot water potential approached -0.8 to -0.9 MPa. Stomatal closure occurred at a shoot water potential between -1.2 MPa to -1.5 MPa. Mortality due to water stress occurred only in response to extended periods of low soil water and was associated with a shoot water potential of below -3.0 MPa. There was variability between seedlings in their potential for survival and growth. Inherently bigger seedlings had a greater capacity for new root growth following planting. New root growth, as well as a greater mass of new roots, was associated with higher shoot water potentials and higher rates of transpiration under conditions of low soil water availability. This indicated that seedling quality, as determined by size, may play a role in sensitivity to water stress.

The field trials, retrospective study and pot trials indicated that the practice of planting with water was not always critical to the survival of *P. patula* seedlings. A simple financial model was developed to estimate whether planting with water represented a cost that could be used as a decision criterion, given certain growth parameters and management scenarios. The data projected by the model were also compared to actual research data for water versus dry planting (and the inclusion of an insecticide in the water). While these comparisons were specific to the parameters included in the model for this study, as well as the results of the research trials used in the benchmarking exercises, the model indicated that; 1) costs for planting with water were likely to be recovered only when no blanking (replacing of dead trees) was carried out, with capital invested at a low return rate (3%), 2) including an insecticide in the water increased the likelihood of cost recovery, and 3) site quality had an impact on the increase in survival required to recover planting method costs, with a greater percentage increase in survival required on lower quality sites. Lower quality sites often have a lower mean annual precipitation (associated with higher rainfall variability), or shallow soils (associated with lower soil water availability) and therefore are also likely to be sites where foresters may want to use water to reduce (drought related) mortality. The impact of site quality is thus also an important factor to include in any decisions regarding planting methods (i.e. using water) and their costs.

Further investigations should be aimed at examining; 1) the interaction of root plug size (as determined by container type) and soil water availability on growth and physiology of *P. patula* seedlings, 2) the methods of grading seedlings within a population to select those that have a high potential for survival and growth, and 3) the effects of soil water availability on the physiology, survival and growth of *P. patula* cuttings, as well as other pine species and hybrids grown in South Africa, such as *P. elliottii*, *P. elliottii* x *P. caribaea* and *P. patula* x *P. tecunumanii*. It is likely that the proportion of forestry regions planted to these hybrids will increase in the future.

Declaration

The experimental work in this thesis was carried out under the supervision of Prof. Norman Pammenter[1] and Dr Keith Little[2].

I hereby declare that this thesis submitted for the degree of Doctor of Philosophy at the University of KwaZulu-Natal, is the result of my own investigation, unless acknowledged to the contrary in the text.

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Publications and presentations related to this research

Details of publications that form part of the research presented in this thesis.

Published reports and papers

Rolando C.A. and Little K.M., 2007. Results from four *Pinus patula* water planting trials in the summer rainfall region of South Africa. *Southern Hemisphere Forestry Journal* 69: 9-17.

Rolando C.A. and Crous J., 2008. Water versus dry planting: A synthesis of three month survival data for 58 *Pinus patula* research trials. *Indian Forester* 133: 1590-1602.

Rolando C.A. and Little K.M., 2008. Measuring water stress in *Pinus patula* Schiede ex Schlect. & Cham. seedlings. *South African Journal of Plant and Soil* 25(1): 56-62.

Rolando CA., 2007. Using a cash flow analysis to estimate the minimum increase in survival required to recover planting method costs for a pulpwood rotation. *ICFR Bulletin Series No. 16/2007*. Institute for Commercial Forestry Research, Pietermaritzburg, South Africa.

Papers to be submitted for publication

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CHAPTER 1

GENERAL INTRODUCTION TO REGENERATION OF *PINUS PATULA* IN SOUTH AFRICA

1.1 INTRODUCTION

Regeneration is the act of renewing tree cover by establishing young trees naturally or artificially (Helms, 1998). Regeneration usually maintains the same forest type and is carried out shortly after the previous stand or forest was removed. The regeneration phase extends from the time of planting until the trees are established which is generally accepted to occur at canopy closure (Helms, 1998). In terms of plantation forest management, decisions made at the time of regeneration are critical to the success of the rotation and commit the forest company to many subsequent actions and investments. Regeneration may therefore afford the greatest opportunities for meeting the company's objectives but also, possibly, for the biggest mistakes. The most critical aspect of regeneration is survival, as this is a key factor in maintaining a target stem density per hectare, avoiding costly replanting operations and also achieving the yield potential of a site (Chambers and Borralho, 1997). Factors related to stand growth and tree uniformity are important in conjunction with maintaining the target stem density.

Following planting, environmental conditions impose some degree of stress on the planted stock (Margolis and Brand, 1990). These include factors such as non-optimum soil temperature (Kaufmann, 1977; Nambiar *et al.*, 1979), air temperature and humidity (Kolb and Robberecht, 1996), and soil water availability (Khan *et al.*, 1995; Généré and Garriou, 1999). Successful regeneration therefore depends on the interaction between the phenological and physiological characteristics of the planting stock and the environmental conditions of the planting site (Burdett, 1990; Margolis and Brand, 1990; Radoglou *et al.*, 2003). Phenology is influenced by the growing environment and is reflected in the structural and physiological characteristics of the planting stock (Burdett, 1990; Livonen *et al.*, 2001; Barnes, 2002; Gazal *et al.*, 2004). The physiology of the planting stock is affected by genetics, past and present growing conditions and this will have a direct impact on the short and long term performance of the trees.

The South African commercial forestry industry is based entirely on species that are introduced to the region (Zwolinski and Bayley, 2001). Growing trees for timber is possible only in areas with sufficient rainfall (>800 mm) and this occurs in a narrow belt extending along the south and east coasts, and in the mountainous regions of the eastern side of the country (Schulze, 1997). This eastern belt, commonly referred to as the "summer rainfall region", occurs between the mountains and the sea extending from the eastern Cape to northern Mpumalanga. In 2006, there were approximately 1.3 million hectares of commercially planted timber, 54% of which was planted to pines either for pulpwood (30%) or structural timber (70%), (DWAF, 2005/06). The major pine species include *Pinus patula* Schiede ex Schlect. & Cham (49.5%), *P. elliottii* Engelmann (28.6%) and *P. radiata* D. Don (5%; winter rainfall regions only). Pines are usually planted at 1111 stems ha^{-1} for saw timber and between 1333 and 1736 stems ha^{-1} for pulpwood production (Zwolinski and Bayley, 2001). Although *P. patula* is the most widely planted softwood species for both pulpwood and saw timber, mortality following planting is of major concern and has the potential to limit future deployment of this species for commercial production of timber.

The complexity of the many principle and interacting factors affecting regeneration success often makes the determination of causes of mortality and (or) poor growth difficult. Never-the-less, there are several key issues critical to a discussion on regeneration, and some of these are described briefly below.

1.1.1 Soil water availability

Water deficits are one of the major causes of regeneration failure following planting (Burdett, 1990; Margolis and Brand, 1990). Following planting, the planting stock must establish root-to-soil contact, and commence water and nutrient uptake in order to survive (Sands, 1984). If the plant does not receive water during the period of new root regeneration, its internal water deficits will increase considerably (Burdett, 1990). Since the metabolic activity of cells is closely related to their water content almost every plant process is affected directly or indirectly by the supply of water. Cell enlargement is dependent on a minimum degree of cell turgor and root and shoot growth quickly cease when water deficits occur (Kramer and Boyer, 1995). In addition, a decrease in water content also inhibits photosynthesis, respiration and other enzyme-mediated processes (Kramer and Boyer, 1995). Once a leaf loses turgidity the stomatal guard cells close, preventing any further intake of carbon dioxide needed for photosynthesis. The ability of a plant to withstand water deficits therefore depends, *inter alia*, on its critical leaf water potential (the potential at which the stomata close) which differs markedly amongst species (Schulze, 1997). A high tissue water potential after planting therefore allows for the onset of a positive cycle of root growth supported by photosynthesis and photosynthesis supported by root growth (Burdett, 1990; Margolis and Brand, 1990). The mutual dependence of root growth and photosynthesis are therefore central to the processes in plantation establishment or regeneration (Burdett, 1990). The characteristics of planting stock that are crucial to survival are most likely to be those affecting plant water status and the capacity for root growth and photosynthesis following planting.

The ability of plants to tolerate water stress, or drought, has been described as drought tolerance (Barnes, 2002). Mechanisms that contribute to drought tolerance can be separated into three groups; 1) stress avoidance mechanisms that limit the occurrence of damaging water deficits, 2) stress tolerance mechanisms that maintain physiological activity as plant water deficits increase, and 3) efficiency mechanisms that optimize the use of limited resources (Livingston and Black, 1987; Barnes, 2002). Plants in the genus *Pinus* tend to be stress avoiders (Richardson and Rundel, 1998). For individual pine species there is evidence of a variety of mechanisms of drought avoidance, including high water-use efficiency, enhanced water uptake through the development of extensive root systems, comparatively high hydraulic conductance through stems and roots per unit leaf area and high capacitance, or water storage (Rundel and Yoder, 1998; Barnes, 2002; Oviedo and Emmingham, 2003). There is also evidence that pines may absorb dew through needles thereby improving leaf-water relations, and potentially affecting survival in very dry soil (Boucher *et al.*, 1995). Exposure of pine planting stock to water stress during the hardening off phase in the nursery has been shown to influence their ability to survive water stress following planting, through a variety of mechanisms (Kaushal and Aussenac, 1989; Burdett, 1990; van den Driessche, 1991). These include an increase in carbon partitioning to roots relative to shoots (Barnes, 2002), affecting osmotic adjustment (Cannell, 1985) and increasing photosynthesis at low water potentials (Seiler and Johnson, 1985). Availability of water, in terms of mean annual precipitation and its affect on soil water availability, is considered one of the major limiting factors to commercial tree growth in South Africa (Schutz, 1990). Despite this there is very little published research on the water relations and drought tolerance mechanisms of major commercial timber species, especially during the regeneration phase.

1.1.2 Root growth

Root growth following planting of stock has been shown to be related to morphological, physiological and environmental factors (Burdett *et al*., 1983). Morphological and physiological factors that have been related to a high capacity for new root growth following planting include; 1) heritability for a high number of first order lateral roots (Kormanik and Muse, 1986), 2) low shoot:root ratio of the planting stock (Larsen *et al*., 1986; Boyer and South, 1989; van den Driessche, 1991), 3) availability of carbohydrates (van den Driessche, 1987; Burdett, 1990; Tinus *et al*., 2000), 4) high "root growth capacity" as determined by a measure of new root growth under controlled conditions (Ritchie and Dunlap 1980), 5) total root weight (South *et al*., 1990), and 6) planting stock size (as measured by root collar diameter, root volume and total weight) (Carlson, 1986; South *et al*., 1990). The ability of planting stock to grow new roots after planting has also been considered as a measure of quality (Nambiar, 1980; Sutton, 1990).

Environmental factors that have been shown to affect root growth following planting include; 1) soil temperature (Nambiar, 1980; Ritchie and Dunlap, 1980; Sutton, 1990; Kramer and Boyer, 1995), 2) soil moisture availability (Ritchie and Dunlap, 1980; Gazal *et al*., 2004), 3) light intensity (Kramer and Boyer, 1995), and 4) photoperiod (Partanen and Beuker, 1999). Seasonal patterns of root growth partly reflect changes in these parameters. All species have an optimum soil temperature range for the development of new roots and planting should be planned to coincide within the season when this occurs. Previous studies on pines have generally indicated that optimum root growth occurs in soil temperatures of 20-25˚C, with growth declining at higher and lower temperatures, especially below 5˚C (Lopushinsky and Max, 1990). In pine seedlings, root zone temperature affects both initiation of new roots and elongation of existing roots (Brissette and Chambers, 1992; Oliet *et al*., 2001).

Seasonal patterns in the root and shoot growth of trees are also known to exist, where alternating allocation patterns to above and below ground biomass occur (Sung *et al*., 1993; McMillan and Wagner, 1995). This pattern is most likely the result of allocation of energy reserves within the plant that may be dependent on genotype (Merritt, 1968; Sword-

Sayer *et al*., 2005) and environmental factors, such as water deficit (Commeau and Kimmins, 1989), soil temperature (Sword-Sayer *et al*., 2005) and soil nutrient status (McMillin and Wagner, 1995). For example, *P. ponderosa* typically has an early season root growth period prior to shoot elongation and then a second peak in root growth following bud set (Jenkinson, 1975). Jenkinson (1975) found four distinct seasonal patterns of root growth of ponderosa pine seedlings based on geographical origin. In a further study, McMillin and Wagner (1995) showed that patterns of biomass allocation during periods of root and shoot growth could also be affected by water stress. Consideration of the timing of water deficits in relation to tree ontogeny and phenology may therefore be important in developing an understanding of the factors promoting survival during regeneration, including that of *P. patula*.

1.1.3 Planting stock quality

The issue of planting stock quality is complex and only some of the major factors will be highlighted in this section. The desirable physiological and morphological traits of quality planting stock change according to the objectives of the organisation or the individual buying or using the stock (South and Mexal, 1984; Mohammed, 1997). Factors that are important include species, provenance, climatological factors, planting site, time of planting and the financial goals of the organization (South and Mexal, 1984; Bayley and Kietska, 1997; Mattsson, 1997). When the goals of management are broad (non-profit or production driven), the range in acceptable criteria will be high. In contrast, when the objectives of management become more specific, the criteria for acceptability will be more rigorous (South and Mexal, 1984). South and Mexal (1984) defined planting stock quality as “the degree to which stock realizes the objectives of management at a minimum cost”.

The evaluation and definition of planting stock quality can be through morphological and (or) physiological measures. The most frequently measured morphological attributes include height, stem diameter, bud diameter and shoot:root ratio (Mattsson, 1997). Physiological measures may include water status (Scholander *et al*., 1965), root growth potential (Ritchie and Dunlap, 1980), electrolyte leakage (McKay, 1992) and chlorophyll fluorescence (Vidaver *et al*., 1991; Mahommed *et al*., 1995; Rolando and Little, 2003). Of the physiology measures, root growth potential is considered the best as it has the potential to indicate when planting stock is resistant to stress, and is simple and easy to conduct (Sutton, 1990). There is, however, some debate as to the relevance of this test to field performance (Simpson and Ritchie, 1997). It is unlikely that any single morphological or physiological parameter is likely to predict field performance following planting and it is generally accepted that an integrated approach, incorporating various measures, is likely to be the most successful (Mattsson, 1997). Whichever method is used, the ultimate definition of quality is dependent on actual survival and growth following planting (South and Mexal, 1984; Puttonen, 1989; Dunsworth, 1997). An ideal test of planting stock quality for operational use would be one that could be; 1) rapidly carried out (yielding results almost immediately), 2) simple in application and interpretation, 3) reliable and non-destructive, and 4) diagnostic (so that the cause of any seedling damage would be indicated), (Mattsson, 1997).

There has been a considerable amount of research on the characteristics of quality stock and the assessment thereof in the United States (Kormanik and Muse, 1986; Tanaka *et al*., 1997; Momen *et al*., 2004), Canada (Dunsworth, 1997; Mohammed, 1997; Sampson *et al*., 1997), New Zealand (Menzies *et al*., 2001) and parts of Europe (Puttonen, 1989; Mattsson, 1997). Much of this research is relevant to stock that has been produced from bare-root nurseries and is therefore not always directly applicable to the South African situation, where all stock is produced in a container nursery system (Zwolinski and Bayley, 2001). In general, the published research has shown that the planting of bareroot stock with larger root collar diameters increases early survival and growth during regeneration, indicating that plant size has a key role to play in planting stock quality (Jinks and Kerr, 1999; South, 2000; Puertolas *et al*., 2003). Many bare-root nursery systems have a grading system based on measurements of root collar diameter whereby planting stock is assigned to different quality classes prior to deployment. For example, South (2000) showed an improvement in early survival and growth of (bare-root) *P. taeda* and *P. elliottii* stock if more than half were selected to have a root collar diameter of more than 5 mm and all were selected to have a collar diameter of greater than 3 mm.

In comparison to research on stock quality for bare-root systems, little work has been carried out on the parameters defining stock quality for plants grown in containers, internationally and particularly, locally (Bayley and Kietska, 1997; Zwolinski and Bayley, 2001; Menzies *et al*., 2001). Difficulties for defining optimum stock are associated with the huge variety of available container types. In a containerised system, there is an optimum "plant quality window" during which the seedling is at an ideal age and size for that particular container type (South and Mitchell, 2006). This will vary between different containers and growing regimes, and must be established for a particular system. A plant that is too young will invariably have a poorly consolidated root plug that may be easily damaged during planting (and subsequently increase the risk of mortality). A plant that is too old (kept in a container for too long) may often have a constrained (root-bound) or abnormal root system that can lead to stability problems and mortality later on in the rotation (South and Mitchell, 2006). In a container, a quality plant can therefore only be raised for a finite period and must be planted during the 'window" when roots have consolidated the volume of the container but have not become restricted. Factors that have been shown to increase survival following planting with stock produced from containers include an increase in container volume, a low index of root-binding, low height to root collar diameter ratio, high root to shoot ratio and root growth potential (Bayley and Kietska, 1997; Mitchell *et al*., 2005a; South and Mitchell, 2006).

The interaction between phenology, water stress physiology and indicators of plant quality of several conifer species have been well documented for plants grown in the United States (Kaufmann 1977; Drew and Ledig 1980; Running 1980; Larsen *et al*., 1986; Kolb and Robberecht 1996; Tinus *et al*., 2000; Cregg and Zhang 2001; Barnes 2002), and Europe (Kaushal and Aussenac 1989; Généré and Garriou 1999; Villar-Salvador *et al*., 1999; Livonen *et al*., 2001; Royo *et al*., 2001). The most common pine species used for forest regeneration in these regions are the temperate species of *P. ponderosa*; *P. contorta*; *P. taeda*, *P. sylvestris*, *P. radiata* and *P. halepensis*. As previously stated, seedlings of these species are generally produced from bare-root nursery systems, with container systems limited to species not suited to bare-root practice (e.g. *P. palustris*). This, together with the fact that most planting takes place in winter, renders the system of pine regeneration used in other, major forestry regions, very different from that used in South Africa. The implication is that while a large body of published research on aspects of plant quality, phenology and water stress physiology exists for pine planting stock, this information is not directly applicable to the major pine species grown for regeneration in South Africa.

1.1.4 The planting operation

The success of regeneration may also be directly influenced by the planting operation, including nursery to field transport and handling (South and Mexal, 1984; Nelson, 1991;

Grossnickle and Folk, 1993; Morris, 1994; Myburgh, 2005), planting season (South and Mexal, 1984; Long, 1991; Radoglou *et al*., 2003) and planting method, including depth of planting and the use of hydrogels, fertilizers and water (South and Mexal, 1984; Long, 1991; van der Schaaf and South, 2003; Gilman and Grabosky, 2004; Viero and Little 2006). The primary objective of any planting operation is to place the planting stock into the ground in a manner that will optimize survival and growth. The chosen method will depend on the type and condition of available seedlings, soil and site characteristics and the intensity of site preparation (Long, 1991; Evans, 1992). Since most of these factors are largely of an applied nature (training and management related) they have received relatively less discussion in the literature, although their impact on survival and growth can be substantial.

An often overlooked phase in the regeneration window is the period between the despatch of planting stock from the nursery to the time it is planted (nursery to field handling and transport) (Myburgh, 2005). Poor management during this period can result in physical damage to the planting stock and induce water and (or) heat stress resulting in an increase in field related mortality (Evans, 1992; Myburgh, 2005). Myburgh (2005) emphasized the need for good supervision and quality control of handling of planting stock during the transport phase to avoid factors that contribute to drying out, wind scorch, exposure to excessive temperature, rough handling and excessive shaking. Myburgh (2005) further suggested these factors could be minimised through the designation of responsibilities to particular individuals involved in the process as well as the use of well designed transport for moving the stock from one location to the next. In South Africa, following despatch from the production nursery, the planting stock is kept at a holding nursery at the plantation where the seedlings are to be planted. The function of the holding nursery is as a short-term storage facility to allow the forester flexibility to choose planting days as dictated by optimum weather conditions. To maintain the quality of the planting stock, the plants should not remain for more than two weeks in the holding nursery. Failure to plant the stock timeously can result in stock that becomes root-bound with associated high shoot:root ratios not conducive to good survival following planting (Myburgh, 2005).

The optimum time (or season) for planting, or “planting window”, varies largely between regions (internationally and locally) and is dependant on factors such as species, planting stock, planting site, seasonal rainfall, local weather conditions, the area that requires replanting and the availability of labour (Long, 1991; Evans, 1992). Weather conditions at the time of, and immediately after, planting can significantly affect survival (Long, 1991; Morris, 1990; Rolando and Little, 2004). The most critical determinants in this regard are those conditions that affect seedling water loss (or promote water retention) such as low soil moisture and high air temperature, relative humidity and wind speed. As limited research has been carried out on the effect of planting time on subsequent survival and growth of pines in South Africa (Morris, 1990; Allan *et al*., 2000; Allan and Higgs, 2000), there is a need for more research leading to the development of stringent guidelines to effectively utilize optimum planting windows. In the summer rainfall region for plantation forestry in South Africa, the rainy season occurs during spring and summer (October to March) which coincides with extremes in high air temperatures (Schulze, 1997). In addition, rainfall can be sporadic during this period, and often occurs as short intense rainstorms. Planting in summer can be associated with high mortality and needs to be carefully timed to coincide with forecasted wet periods and cloudy days. In the sub-tropical and coastal region of Zululand, planting of *Eucalyptus* species is carried out during the winter months (April to October), (Viero, 2000). Although, this region receives some precipitation during the winter months, the cooler temperatures and lower evapo-transpiration rates allow for better establishment and lower post-planting stress (Viero, 2000).

Aspects related to planting method have been extensively researched for bare-root pines planted in the United States (Long, 1991; Brissette *et al*.,1991). Worldwide, there is also an increasing number of hand held and tractor drawn implements that can be used for making planting holes, inserting trees and packing soil back around the roots. Designs vary widely and site conditions and economics are the major factors influencing equipment selection (machine planting is not used in South Africa), (Long, 1991; Evans, 1992). Regardless of the method used, successful planting still depends on the ability of the roots of the planted trees to regain contact with the soil so that the uptake of water and nutrients can resume (Burdett, 1990). Planting methods used elsewhere differ markedly from that in South Africa, largely due to differences in planting stock (bare-root elsewhere versus container stock in South Africa), site preparation (intensive preparation elsewhere versus minimum tillage used in South Africa) and availability of labour South Africa. Almost all silvicultural operations in South Africa are manual. The planting hole is made into an area of manually loosened soil (a planting pit) with a hand held trowel, or mattock, and the seedling is placed into the hole before back-filling with soil to secure the root plug (Smith, 2000). In South Africa, little research has been conducted on the effect of planting implement and placement of stock on survival.

Planting depth is defined as the distance between the root collar and the soil surface (South, 1999), with the maximum planting depth a function of the seedling size (bigger seedlings can be planted deeper in the soil profile) and depth of planting hole (shallow planting holes will restrict the depth to which the root plug can be placed). Several researchers have tested the effect of planting depth on survival (McGee and Hatcher, 1963; Schwan, 1994; van der Schaaf and South, 2003; Gilman and Grabosky, 2004) and in most cases it has been found that deeper planting increases survival. Better survival of deeper planted stock has been related to a higher soil water content deeper in the pit as well as the development of adventitious roots in some species (Sutton, 1966). As a result, deeply planted stock have been found to tolerate short term dry periods better than those planted with the root collar nearer the surface (van der Schaaf and South, 2003). Besides one study by Donald (1970) on bare-root pine seedlings, no other published literature on the effect of planting depth on subsequent survival and growth of any species grown in South Africa could be sourced.

1.1.5 Pests and diseases

Mortality of planting stock during regeneration due to pests and diseases is widespread. The timeous treatment of seedlings with appropriate pesticides at planting, combined with the practice of alternative (for example: cultural) pest management recommendations, has been found to improve post-planting survival (Hodges, 1964; Haywood and Tiarks, 1994; Hallgren and Ferris, 1995; Brissette *et al*, 1996; Salom, 1997; Lindelow and Bjorkman, 2001). Haywood and Tiarks (1994) reported a significant increase in survival, for up to ten years, of *P. elliottii* seedlings treated at planting with the systemic fungicide triadimefon, effective against several plant diseases including fusiform rust, the most destructive disease of *P. elliottii*. The pales and pitch-eating weevils, *Hylobius pales* Herbst and *Pachylobius picivorus* Germarb, are the most serious insect pests of pine regeneration throughout the southern United States (Lynch and Heddon, 1984; Salom, 1997). Similar to the bark beetle *Hylastes angustatus* Herbst in South Africa, these weevils feed on the bark of seedlings during spring and autumn, with the intensity of their impact decreasing with increased time between clearfelling and regeneration. Management to reduce mortality includes delaying replanting for one to two years after harvest or treating the seedlings with an insecticide either before or after planting (Nord *et al.*, 1978).

A considerable amount of applied research on pest management during regeneration has been conducted on behalf of the South African forestry industry (Morris, 1990; Atkinson and de Laborde, 1993; Atkinson and Laing 1996; Atkinson and Govender 1997; Atkinson, 1999; Allan and Higgs, 2000; Mitchell *et al.*, 2004; Rolando and Allan, 2004; Crous, 2005). The majority of these studies have shown that the application of a pesticide (usually with water) to the planting pit at planting increases survival of pine seedlings over those planted without. The magnitude of the response often coinciding with season of planting, possibly related to when the insect pest or disease outbreaks occur, or method of harvest residue management (Allan and Higgs, 2000; Rolando and Allan, 2004). Analysis of thirty pine trials planted in the summer rainfall region showed an average of 4, 14 and 9% increase in survival at twelve months in response to the application of an insecticide, fungicide or both during planting (Rolando, 2006). Common insect pests and diseases of pine seedlings during regeneration in South Africa include: *Hylastes angustatus* (pine bark beetle); *Agrostis* spp. (cut worm); *Rhizina undulata* Fries; *Fusarium circinatum* Nirenburg and O Donnell and *Diplodia pinea* (Desm.) Kickx. (Germishuizen, 1984; Swart *et al.*, 1985; Kirsten *et al.*, 2000; Wingfield and Roux, 2000; Coutinho *et al.*, 2007). Currently there are no registered fungicides for use on pines following planting. This is of serious concern for the successful regeneration of pines, particularly *P. patula*, in the summer rainfall region of South Africa.

1.1.6 Economic considerations of regeneration

Decisions made at regeneration will depend on a combination of management objectives and regeneration alternatives available for the site and the economic costs and returns on the investment (Uys, 2000). Forest companies often have limited capital to invest in timber production and therefore may choose the method of regeneration that will minimize costs and maximise returns. Together with biological knowledge, a sound application of economic principles is also crucial to ensure successful regeneration. Unfortunately, decisions made at regeneration are often dictated more by economic than biological criteria (Cubbage *et al.*, 1991). All economic analyses of forest management and regeneration decisions rest on underlying biological productivity, where basic input and output relationships relate the cost of production to the quantity produced, which for forestry usually relates to stand yield (measured as utilizable underbark volume in m^3 ha^{-1}) (Cubbage *et al.*, 1991). For forestry, the inputs affecting stand yield include harvesting methods, genotype, planting stock, planting density, soil and site factors, methods of competition control and pruning and thinning operations in a sawtimber stand (Bredenkamp, 1980; Donald, 1987; Burger and Jamnick, 1995; South *et al*., 2001; Dean and Chang, 2002; Little *et al*., 2002; South and Rakestraw, 2002). The biological effects of these factors on growth, and their cost, must be understood for economic analyses of regeneration alternatives and subsequent yields (Cubbage *et al.,* 1991). While the details of all the above economic assessments and their application are beyond the scope of this study, regeneration costs are an important consideration to any discussion on alternative methods of regeneration and their implications in terms of success. It is generally recognized, in current financial and forestry literature, that the most acceptable method for assigning values to long-term projects such as forestry is discounted or compounded cash flow analysis (Klemperer, 1996; Cubbage *et al*., 1991; Uys, 2000). The characteristic which distinguishes this technique from others is the recognition that money has a time value and the extended rotation lengths associated with timber production dictate the importance of time (Uys, 2000).

Besides studies conducted by Donald (1986) and South *et al*. (2001) in the eastern Cape region of South Africa, there is little published literature examining the costs of different methods of pine regeneration and the economic implications for the South African pine timber industry. This, despite many questions as to the methods of regeneration that could be used to increase the current poor survival of pines, particularly that of *P. patula*; including the critical examination of planting density, seedling quality, blanking or the use of hydrogels, fertilisers and (or) pesticides during regeneration. This may be reflection of a poor combined understanding of biological and economic considerations. It may also be that the demand for timber at the mill has continued to exceed growing costs, such that any methods of regeneration perceived to contribute to higher yields have been favoured.

1.1.7 Basic and applied research

Research is carried out in order to qualify and quantify unknown variables through scientific study and involves a critical course of investigation. Two types of research are recognised, and include basic (or fundamental) and applied research. The distinction between basic and applied research is sometimes vague, despite the frequent use of these terms in science studies and science policy. In most cases it is based on such pragmatic factors as the knowledge and intentions of the investigator or the type of research institute (Niiniluoto, 1991).

Traditionally, basic research has as its primary objective the advancement of knowledge and the theoretical understanding of the relations among variables. It is exploratory and often driven by the researcher's curiosity, interest, or intuition (Segerstedt, 1983). It is often conducted without any practical end use in mind, although it may have unexpected results pointing to practical applications. The terms "basic" or "fundamental" indicate that, through theory generation, basic research provides the foundation for further, sometimes applied, research. Long term progress in research is usually associated with basic research as there is an effort to advance knowledge and predictive understanding. For regeneration this would include investigations into plant physiology, soil physics, nutrition, plant pathology and entomology. Basic research differs from applied research, in the context of regeneration, in that; 1) its is undertaken to understand a phenomenon, rather than solve an immediate problem, 2) the results of the research may not be immediately applicable to users, and 3) the research itself is not conducted in close consultation with forest managers (Wagner, 1993).

Applied research is carried out for practical purposes, through the application of scientific and mathematical knowledge for the solution of practical problems, often respecting economic principles (Zemanek, 1983). Applied research seeks to develop concepts, techniques, methods, tools or products that are directly applicable to improving practices, in this case, methods of regeneration. In the context of forestry, applied research is generally conducted in close consultation with forest managers, particularly where it is associated with improving silvicultural practices in the short term (Wagner, 1993).

Traditionally, basic research was considered as an activity that preceded applied research, which in turn preceded development into practical applications. Recently, these distinctions have become much less clear-cut, and it is sometimes the case that all phases occur concurrently. The present study is a mixture of both basic and applied research. This is a function of the commercial industry for which the research was conducted, as well a reflection of the need for an advancement in the understanding of the scientific factors driving the survival and growth responses that were observed in the applied studies. Without basic research there is a danger of repeating empirical (applied) trials generating results that can only, ever be specific to the site and management conditions under which they were conducted.

1.2 REGENERATION OF *PINUS PATULA*

1.2.1 Description of *P. patula* as a commercial timber species

Pinus patula occurs naturally in the fog and cloud belt of the mountainous regions of Mexico at elevations between 1500 and 3100 m (Dvorak *et al*., 2000). The climate is temperate throughout most of the natural range, where annual precipitation varies from 1000 to 2500 mm with most of the rainfall occurring in summer (June to October). Average daily temperatures range from 10 to 18˚C, with night time winter temperatures as low as -9˚C. In its natural environment *P. patula* is described as an aggressive pioneer species, relatively disease free, that regenerates rapidly when seeds fall on the exposed mineral soils in forest gaps (Dvorak *et al*., 2000). Approximately 1.0 million hectares of *P. patula* have been planted worldwide, predominantly in southern and eastern Africa and to a lesser extent in the highlands of western South America. *P. patula* requires deep, well drained soils and grows best in areas above 1000 m altitude, at latitudes 18˚ to 30˚ and above 2200 m near the equator (Dvorak *et al*., 2000). The species is said to be moderately drought tolerant when mature and the recommendation is to plant in areas that receive more than 850 mm of annual precipitation (Dvorak *et al*., 2000).

Pinus patula was first introduced to South Africa, from Mexico, in 1907 (Poynton, 1961) and has subsequently become one of the most important softwood timber species in South Africa (DWAF, 2005/06). Sites suitable for *P. patula* in South Africa occur in the mist belts of the eastern highlands between 1200 and 1650 m altitude (Dvorak *et al*., 2000; Morris and Pallet, 2000). Rainfall should be greater than 850 mm per year with no more than four months with less than 25 mm precipitation. Average monthly temperatures should range from 5 to 23˚C (Dvorak *et al*., 2000).

Generally little distinction is made in South Africa between the terms pine regeneration and pine re-establishment, where both terms are used interchangeably and refer to the replanting of a recently harvested site with tree seedlings (Hinze, 1993). Minimum site preparation is carried out prior to the planting of pine in South Africa, and normally includes the preparation of an adequate planting pit combined with pre-planting weed control (Smith, 2000; Little and Rolando, 2001). Pitting is the preparation of a planting position and is carried out to improve the physical environment into which the tree seedling is planted. The planting pit is manually made, with a diameter of between 25 to 40 cm and depth of 20 to 30 cm (Smith, 2000). Most planting of pines in the summer rainfall region occurs between September and May when the soil is moist and rain can be expected (Hinze, 1993; Viero, 2000). Planting methods differ widely between commercial forest companies where there are also large differences in the use of water, hydrogels and fertilizers at planting.

1.2.2 Factors affecting regeneration of *P. patula* in South Africa

In South Africa, regeneration of pines is based almost entirely on planting stock raised from seed grown in container nurseries (Zwolinski and Bayley, 2001). There is limited deployment of hybrid cuttings and research into vegetative propagation of clones (*P. patula*) and hybrids (including *P. elliottii* x *P. caribaea* and *P. patula* x *P. tecunumanii*) and their deployment is ongoing (Mitchell *et al*., 2005b). Large scale deployment of family[1] cuttings of *P. patula* has not yet occurred, largely due to rooting problems when in the nursery, as well as a high incidence of *F. circinatum* in the hedged plants (parent stock) (Dvorak *et al*., 2000). Most of the nurseries producing *P. patula* seedlings are located in the warm to cool temperate regions of the summer rainfall region and production continues throughout most of the year. A wide variety of small containers, ranging between 36 cm^3 and 80 cm^3 in cavity volume, and 49 and 128 cavities per tray, are used for seedling production (Zwolinski and Bayley, 2001). Besides one published study (South and Mitchell, 2006), research to identify the optimum “plant quality window” for different container types is generally lacking, and this, together with a lack of a quality grading system, means that seedlings of poor quality and small size may be used for regeneration. The general consensus amongst foresters and nursery managers is that smaller seedlings survive better than larger seedlings (Zwolinski and Bayley, 2001). This is frequently linked to root malformations of larger planting stock that has been left in small containers too long, a condition locally referred to as “over-prime” or “root bound” (Bayley and Kietzka 1997; Zwolinski and Bayley 2001; South and Mitchell, 2006). Since nurseries are

[1] A family is a group of closely related genotypes usually, half siblings (one parent in common) or full siblings (both parents common), (Mandal and Gibson, 1998).

generally considered as “cost centres”, technology is often aimed at minimizing production costs, a factor also likely to contribute to the use of higher density, smaller volume containers (Zwolinski and Bayley 2001). While the benefits associated with planting larger stock have been shown for a bare-root system in South Africa (South and Zwolinski 1993), equivalent research for a container system is lacking. Bayley and Kietzka (1997) concluded that *P. patula* survival could be improved through identification of the best time of year and conditions for planting, as well as improving stock quality. Since then, some research, including that presented in this study, has been conducted aimed at meeting the requirement of identifying planting windows (Mitchell *et al*., 2005b; Rolando and Little, 2004), though comprehensive research into improving planting stock quality has yet to be undertaken.

Continued high mortality (sometimes as high as 20 to 50%) during regeneration with *P. patula*, and the implications for future pine timber yield, is of concern in South Africa (Crous, 2005; Rolando and Little, 2005). Several applied and empirical studies have been conducted since the early 1990’s to gain an understanding of the factors causing mortality of *P. patula* seedlings. This research indicated that heat and water stress, pests and diseases and the management of the harvest residues were major determinants of mortality (Morris, 1990; Bayley and Kietska, 1997; Allan and Higgs 2000; Allan *et al*. 2000; Rolando and Allan 2004; Rolando and Little, 2004; Crous, 2005; Mitchell, 2005b; Rolando and Little, 2005; Rolando, 2006). In addition, some of this research indicated that elevated air temperatures during the summer, together with high levels of harvesting residues, affected air temperature in the vicinity of the seedlings such that mortality occurred (Allan and Higgs, 2000). More recently, the incidence of *F. circinatum* in all South African pine nurseries has motivated research into the impact of this fungus on survival during regeneration, as well as studies aimed at understanding the epidemiology of this disease (Crous, 2005; Coutinho, *et. al.*, 2007).

Due to the empirical nature of much of the past research, it has been difficult to accept or refute the stated causes of mortality, as well as gain an understanding of the interaction between site, environment, plant growth and physiology. This presented a need for a more fundamental approach in the research to understand factors contributing to low survival of *P. patula*. More specifically, it was recognized that quantification of certain components of the micro-environment (including soil and air temperature and soil water and physical properties) surrounding the seedling at planting, and the physiological response of the plant to these factors was needed to fully understand the conditions and (or) physiological factors that negatively affected early survival and growth of *P. patula* in South Africa (Rolando *et al*., 2003; Rolando and Little, 2004). This was coupled with a general lack of international studies, investigating the morphological and physiological characteristics required for *P. patula* seedlings to survive and grow at any particular site, (Rundel and Yoder 1998; Oviedo and Emmingham, 2003).

1.3 OBJECTIVES OF THIS STUDY

There are likely a number of inter-related factors that affect the success of regeneration with *P. patula*, including planting stock quality, access to water and nutrients, pest and disease attacks and planting weather conditions. However, early survival and growth of tree plantations in South Africa is often hampered by insufficient water supply (Roberts, 1994; Zwolinski, 1997; Viero and Little, 2006). Seedlings can die irrespective of water availability if their physiological or morphological properties deteriorate during production, transport or planting operations. However, high quality seedlings and appropriate regeneration procedures cannot prevent post-planting mortality where water supply is inadequate.

Water stress is considered one of the major causes of regeneration failure (Burdett, 1990) and also one of the most limiting factors to tree growth in South Africa (Schutz, 1990; Roberts, 1994). To counter this, water planting (application of water into the planting hole at the time of planting) of *P. patula* seedlings has been used commercially, to reduce post-planting water stress and buffer against potentially extreme weather conditions immediately after planting (Morris 1994; Allan *et al*., 2000; Oscroft *et al*., 2000; Rolando and Little, 2004). However, the primary function of the water, as well as its success in increasing survival at regeneration, has never been critically assessed. There is also no published research on the water relations and drought tolerance mechanisms of *P. patula* during the regeneration phase, particularly during the three months after planting. Since the use of water in the planting operation is costly, it was essential that the benefits of water planting, in terms of seedling physiology and plantation management (economic), were quantified. This fact, together with questions raised by the industry as to the role of water during the planting operation, initiated the series of studies conducted for this thesis. The situation also represented an opportunity to conduct more fundamental studies aimed at understanding the interactive effects of site, environment and plant water stress in *P. patula* seedlings.

The overall aim of this study was therefore to understand the role of water during regeneration of *P. patula* from both a plantation management and tree physiology perspective. Specifically, the main objectives were to:

1. Establish benchmark physiological data for *P. patula* during regeneration.
2. Determine whether planting with water increased survival, and if so assess;
    a. the season and region where a response was most likely to occur,
    b. the function of the water in the pit and its effect on plant-soil interactions, and
    c. the quantity of water required to effect a positive survival response.
3. Investigate the interaction between planting stock quality (as determined by measures of plant size) and soil water availability and the effect on survival, growth and physiology of *P. patula* seedlings. This included;
    a. the determination of the variability in the growth and physiology in response to changes in soil water availability between different seed sources (families),
    b. the quantification of changes in shoot water potential, stomatal conductance and transpiration in response to changes in soil water availability,
    c. the determination of critical water stress thresholds, and
    d. the investigation of early changes in seedling morphology and the relationship to measurements of water stress, i.e. determine the relationship between measurable morphological attributes and an ability to develop roots and tolerate water stress.
4. Develop an understanding of the financial implications of using water in the planting operation for *P. patula.*

1.3.1 Research Strategy

To provide both a practical and comprehensive understanding of the role of water in regeneration of *P. patula*, and to ensure relevance to the commercial forest industry, it was necessary to structure the research to meet objectives that were both applied and fundamental. Different research methodologies were used that included applied field trials, multivariate methods, controlled environment studies as well as an economic assessment and included:

1. Four applied field trials to examine practical issues related to the use of water in the planting operation for *P. patula*. These trials addressed the interaction of site, season and planting method and their effect on early survival and growth (Chapter 2). Comprehensive data related to macro- and micro-environment conditions were collected and this facilitated an understanding of the results allowing for extrapolation to other sites and conditions.
2. A combined analysis of 58 *P. patula* trials planted between 1990 and 2005 that included both water planted and dry planted treatments (a retrospective study). Multivariate methods were used to determine trends in survival in response to the application of water at planting as affected by region of planting, post-planting conditions and season of planting (Chapter 3).
3. Three controlled environment and intensively measured pot trials. These trials were used to gain a fundamental understanding of the effect of soil water availability on post-planting growth and physiology of *P. patula* (Chapters 4-6).
    a. The first trial was a pilot trial, carried out to determine the physiological data suited to our purposes.
    b. The second and third trials were aimed at answering questions related to soil water availability, growth and physiology.

   The understanding gained from these three trials was used to interpret results observed in the field trials and retrospective study.
4. The development of a simple financial model aimed at estimating the minimum increase in survival that would be required to recover the costs of using water (or any other planting method) in the planting operation using various site and management factors (Chapter 7).

Since relatively little is known about the physiology of *P. patula* seedlings in general, this research also contributed to an increase in the understanding of the survival and growth of this species during regeneration. In addition, the applied nature of aspects of the research will facilitate the improvement of current nursery and silvicultural practices for *P. patula* regeneration in South Africa.

Since most of the research presented in this thesis has been published, or is currently in review, the papers have been presented in their published format and the relevant literature and specific objectives are therefore detailed in each chapter. However, as the papers have been published separately (and not integrated as a thesis), there is some repetition in the introductory literature that was necessary for publication. To avoid further unnecessary repetition of literature, this introduction has been used to integrate the study into the broader scientific perspective, provide an indication as to its relevance to the South African forestry industry, as well as outline the strategic research approach necessary to meet the defined objectives. The specific objectives of each paper are addressed in more detail in the relevant chapters/papers. The final chapter (Chapter 8) is a synthesis of the results presented in each chapter. The significance of the results and their implications for future research are highlighted.

CHAPTER 2

RESULTS FROM FOUR *PINUS PATULA* WATER PLANTING TRIALS IN THE SUMMER RAINFALL REGION OF SOUTH AFRICA

ABSTRACT

Planting with water is used by some forestry companies in South Africa to reduce post-planting water stress. Four trials were implemented to test the response in survival of *Pinus patula* to water applied at planting. Two trials each were situated in the KwaZulu-Natal Midlands and Mpumalanga escarpment. The first trial at each site was planted in spring (October) and the second in summer (February). Watering treatments consisted of different quantities of water used in the planting operation and included 0.5 litres, 2 litres, 4 litres and no water. At all sites the planting treatment affected the depth at which the seedlings were planted. Only at the spring planted trial in the KwaZulu-Natal Midlands was survival of the dry planted seedlings significantly lower than that of the seedlings planted with water, at 90 days after planting. This may have been due to low rainfall during the week before and two weeks after planting, or the small size of the seedlings. Application of 0.5 litres of water to the planting pit at this trial was sufficient to increase survival to a level equivalent to that where 2 or 4 litres of water was used, yet increased soil moisture only in the area immediately surrounding the seedling. Planting with water had no effect on early tree growth. Future research should aim to investigate the importance of seedling quality as well as method of application of water to the planting hole on post-planting survival.

2.1 INTRODUCTION

In South Africa, high post-planting mortality of *Pinus patula*, the predominant softwood species planted at higher altitudes in the summer rainfall region, is a major problem and research to increase post-planting survival is ongoing (Morris, 1990; Bayley and Kietzka, 1997; Allan and Higgs, 2000; Mitchell *et al*., 2004; Crous, 2005; Rolando and Little, 2005). The planting of these sites to *P. patula* is carried out predominantly between September and March, when most of the annual rainfall occurs. Intensive planting schedules, combined with unpredictable rainfall, often forces foresters to plant during hot and dry periods that occur during these months, or alternatively to extend planting into autumn (late season) when soil water availability is decreasing. Planting with water is currently used by some commercial forestry companies to reduce post-planting water stress as well as to buffer against potentially adverse weather conditions (Morris, 1994; Nelson, 1995; Viero *et al*., 2000). A number of factors complicate an assessment of the viability of planting with water. Firstly, the use of and (or) method of application of the water varies widely between regions and commercial forestry companies. Some companies have a "no water" policy, others always plant with water, while some apply water only when planting conditions are considered adverse (hot weather, dry conditions). Company practices also vary in terms of the quantity of water used (one to five litres), as well as the method of application (before planting, after planting, or as a split application), (ICFR, 1995; 1996a; 1996b; Atkinson and Govender, 1997; Viero *et al*., 2000). Past research shows that the application of water when planting *P. patula* seedlings does not always increase survival over that of planting without water (dry planting) (Atkinson and Govender, 1997; ICFR and Mondi Forests, 1997; Allan *et al*., 2000; Rolando and Little, 2004), raising questions as to the role of water in the planting operation.

As part of a series of studies aimed at understanding the affects water may have in the planting operation four trials were initiated to determine the;

- effect of the quantity of water applied at planting on survival and growth,
- impact of planting with water on pit soil moisture and temperature,
- effect of air temperature and rainfall on survival (up to one year),
- impact of planting with water on planting depth.

2.2 MATERIALS AND METHODS

2.2.1 Description of trials and treatments

Two trials each were situated in the KwaZulu-Natal Midlands and Mpumalanga escarpment, at the Linwood and Hebron plantations (Table 2.1). These sites differed in terms of mean annual temperature (MAT) and mean annual precipitation (MAP) as well as soil type, with the soil at Linwood having higher clay and organic carbon contents. The first trial at each site was planted in spring (October) and the second in summer (February). Based on current, regional local and long-range weather forecasts (South African Weather Service[1]) the timing of planting was scheduled to coincide with a period when rainfall was unlikely to occur. Seedlings for all four trials were obtained from local nurseries. Planting pits were prepared with picks, and the trees at Hebron planted at a 3.5 x 3.5 m spacing (816 stems ha^{-1}) and those at Linwood at a 2 x 3 m spacing (1667 stems ha^{-1}).

Table 2.1. Details of the four *P. patula* water planting trials planted in spring and summer in the summer rainfall region of South Africa.

Plantation and region	Season	Date planted	Soil physical properties (%)				Site characteristics			
			Sand	Clay	Silt	OC	Altitude (m asl)	MAT (˚C)	MAP (mm)	Harvest residues
Linwood KwaZulu-Natal Midlands	Spring	22/10/03	6	67	27	7	930	15.7	1300	Chopper-rolled
	Summer	03/02/04								
Hebron Mpumalanga	Spring	16/10/03	48	38	14	3	1200	17.6	1200	Broadcast
	Summer	10/02/04								

Each trial had four treatments replicated five times and arranged in a randomized complete block design. The treatments included the application of 0.5 litres, 2 litres, 4 litres of water, and no water (dry plant). Where 0.5 litres of water was used, the water was poured into the planting hole before placing the seedling and covering the root plug with soil. Where 2 litres or 4 litres of water was used, half of the water was poured into the planting hole before placing the seedling and half poured onto the area around the base of the seedling immediately after covering the root plug with soil. Five labourers (planters) were used at each trial with the seedlings planted by each individual tracked to determine the impact of each planter on initial seedling height and subsequent survival. Each treatment plot consisted of 5 x 5 trees. Prior to planting, a chemical weeding with glyphosate was carried out, after which the experimental areas were weeded as per the commercial schedule for

[1] www.weathersa.co.za

the compartment. Weekly rainfall and daily air temperature (1.3 m above ground) and relative humidity were recorded at each site prior to, at and subsequent to planting. Vapour pressure deficit was derived from daily maximum air temperature and relative humidity (Unwin, 1980).

2.2.2 Measurements

Seedlings

To characterize the seedlings planted at each site, measurements of seedling height (from the top of the root plug to the growing tip) and root collar diameter were made on 30 randomly selected seedlings before planting. Samples of seedlings from each batch were also sent to a pathology laboratory[2] for the detection of the pathogen *Fusarium circinatum*. Seedling survival was assessed at 30, 60, 180 and 365 days after planting and seedling height (Ht in cm: from the ground to growing tip) and groundline diameter (Gld in mm) were measured immediately after planting (0) and then at 90, 180 and 365 days after planting.

Pit soil moisture

A Delta-T Theta Probe, type ML2, (Delta-T Devices Ltd) was used to measure pit soil moisture content in the top 0.06 m, initially on the day of planting, and then every two to three days thereafter for the first 14 days. The Theta Probe data recorded at all trials were calibrated using the method described by Little *et al*. (1996). To determine changes in soil moisture content across the pit, readings were taken at distances of 0.05, 0.10 and 0.15 m from the seedling in the first five pits of each treatment plot.

Soil and air temperature measurements

Soil temperature measurements were made at a depth of 0.10 m below the soil surface (in the root plug zone) in four pits of each treatment in the summer planted Linwood trial. These temperature measurements were taken with copper-constantan (Type T) thermocouples connected to a Campbell Scientific CR10x datalogger which used a AM416 Multiplexer to increase the number of thermocouples that could be measured (Campbell Scientific, 1997). A 10TCRT thermocouple was used for the reference temperature with a maximum measurement error of 1.66°C (Campbell Scientific, 1997). One thermocouple

[2] Tree Diagnostic Clinic, FABI, University of Pretoria, Pretoria, 2000

was placed at each measurement point and the temperatures that were logged every hour were an average of measurements taken every two minutes.

Air temperature at a height of 0.10 m above the soil surface and adjacent to the seedling (at 0.10 m to 0.15 m from seedling) were measured on seedlings (n=16) at the summer planted Linwood trial. The measurements were made with H8 Onset Hobo® temperature loggers (Onset Computer Corporation) housed, one each, in gill radiation shields.

2.2.3 Analyses

Analyses of variance (ANOVA) was used to detect differences in survival and growth following planting. Only if the *F*-test was significant were the means further investigated using the least significant difference statistic. Bartlett's test was used to check the assumption for homogeneity of variance required for a valid ANOVA to be performed (Mead and Curnow, 1983). Where necessary percentage survival data were arcsin transformed before analyzing, and detransformed percentage survival is shown in the text and tables. To test the effect of individual planters on initial seedling height (measured on the day of planting), each seedling was scored for planter (1-5) and this score was used as a covariate in the analysis. Unfortunately, herbicide applied during a weeding operation affected survival in both the spring and summer plantings at Hebron and as such these trials had to be abandoned at 60 and 90 days after planting respectively. Results up to the time of herbicide damage have been presented.

2.3 RESULTS AND DISCUSSION

2.3.1 Early growth and survival

There were significant differences between treatments at all four trials in terms of seedling height when measured immediately after planting, indicating that the planting treatment affected the depth at which the seedlings were planted (Table 2.2 and Table 2.3). Seedlings planted with 2 or 4 litres of water were generally planted deeper than those planted with 0.5 litres or dry planted (Table 2.3). Besides this, no further significant differences in measures of height or groundline diameter were detected. On average, seedlings were planted with the root collar diameter 3.5 cm below ground, with the smallest seedlings (in terms of shoot length before planting) planted at the Linwood trial in spring (Table 2.3). When used as a covariate, planter had a significant effect on height at planting at all trials,

Table 2.2. Summary ANOVA of mean squares for initial height (Ht) and survival at four *P. patula* water planting trials established in spring and summer in the KwaZulu-Natal Midlands (Linwood) and Mpumalanga (Hebron). Planter was used as a covariate to detect any differences between planters in terms of planting depth.

		Linwood: spring			Linwood: summer			Hebron: spring			Hebron: summer		
Source of variation	df	Ht (cm)	Survival (%) 30 days	Survival (%) 90 days	Ht (cm)	Survival (%) 30 days	Survival (%) 90 days	Ht (cm)	Survival (%) 30 days	Survival (%) 60 days	Ht (cm)	Survival (%) 60 days	Survival (%) 90 days
Rep	4	85.4	70.6	78.0	173.4	10.0	33.6	19.9	0	25.9	29.8	39.0	10.0
Treat	3	689.7**	37.3^{ns}	163.2*	196.4**	8.9^{ns}	165.9^{ns}	555.7**	0^{ns}	44.2^{ns}	20.4**	143.1*	187.9**
Covariate	(1)	90.8**			97.3**			144.3**			15.6*		
Residual	12 (491)	18.7	33.4	49.6	5.3	14.4	57.4	7.4	0	25.2	2.8	31.2	18.5
Total	19 (499)												
Grand mean		3.8	99.3	95.9	10.3	99.9	98.6	12.8	100.0	99.8	9.0	97.2	96.0
CV (%)		30.0	6.8	9.0	22.0	4.3	5.1	20.2	0	2.2	18.7	7.2	5.0

Note: ^{ns}, *and ** indicate not significant, significance at $p<0.05$ and significance at $p<0.01$.

Table 2.3. Seedling height (cm) measured before and after planting at four *P. patula* water planting trials established in spring and summer in the KwaZulu-Natal Midlands (Linwood) and Mpumalanga (Hebron).

Trial	Height[1] before planting (cm)	Height[2] (cm) after planting[3] dry	0.5 litre	2 litre	4 litre	Mean	Standard error of mean
Linwood: spring	7.1	4.0b	4.8c	3.1a	3.5a	3.8	0.46
Linwood: summer	14.6	10.4b	12.1c	9.8b	9.2a	10.4	0.53
Hebron: spring	14.9	12.2b	14.4c	14.6c	10.1a	12.8	1.10
Hebron: summer	13.1	9.6b	8.9a	8.9a	8.6a	8.9	0.46

[1] Height measured from top of root plug to growing tip.
[2] Height measured from ground to growing tip.
[3] Within each row, numbers followed by different letters are significantly different at $p<0.05$, using the *lsd* statistic.

indicating that each planter played an important role in terms of the depth at which the seedlings were planted (Table 2.2 and Table 2.4). However, the covariate, planter, was no longer significant from the second measurement onwards. In a trial to determine factors affecting survival during late season planting of *P. patula*, Morris (1994) found that individual planter skill was one of the main determinants of post-planting survival. No *F. circinatum* was detected in any of the samples sent to the pathology laboratory.

Table 2.4. Ranking of planters 1 to 5 according to average height of seedlings after planting at each of the four trials. Values in parentheses: 1 = planted the deepest; 5 = planted the shallowest.

Trial	Planter 1	Planter 2	Planter 3	Planter 4	Planter 5
Linwood: spring	4.4 cm (5)	4.3 cm (3)	2.8 cm (1)	3.4 cm (2)	4.4 cm (4)
Linwood: summer	11.0 cm (5)	10.5 cm (4)	10.1 cm (3)	9.9 cm (1)	10.0 cm (2)
Hebron: spring	11.9 cm (1)	12.9 cm (3)	12.6 cm (2)	13.0 cm (4)	13.7 cm (5)
Hebron: summer	9.3 cm (5)	9.2 cm (4)	8.6 cm (1)	9.1 cm (3)	8.7 cm (2)

Survival of the seedlings in the dry planted treatment at the Linwood (spring) trial was significantly lower than that in all water treatments (Figure 2.1a). This difference was significant from 90 days after planting (Table 2.2), when the average difference in survival between the trees planted with water versus the dry planted trees was 12%. Survival of all three treatments planted with water were similar (> 90%), 365 days after planting (Figure 2.1a). The Linwood (spring) trial was planted on a very hot day (maximum air temperature 29.1˚C) with a number of hot, dry days during the 14 days following planting (Table 2.5). In addition, there was very little rain seven days prior to, and immediately after, the planting of the trial (Table 2.5). At the Linwood (summer) trial (Figure 2.1b) there were no significant differences in survival between treatments during the first 90 days after planting, with survival greater than 90% for all treatments at 365 days after planting (Figure 2.1b). Post-planting temperature and rainfall were similar to that which occurred during the spring trial, with more rainfall occurring during the seven to 14 days following planting (Table 2.5). Vapour pressure deficit was higher during the seven days after planting at the spring trial, than that during summer, and very little rainfall occurred. Although the vapour pressure deficit was higher during the seven to 14 days after planting at the summer trial, 56 mm of rainfall occurred during the second week (Table 2.5; Figure 2.2). The lower vapour pressure deficits during the first week of the summer trial, as well as the higher rainfall during the second week, may have alleviated severe water stress in

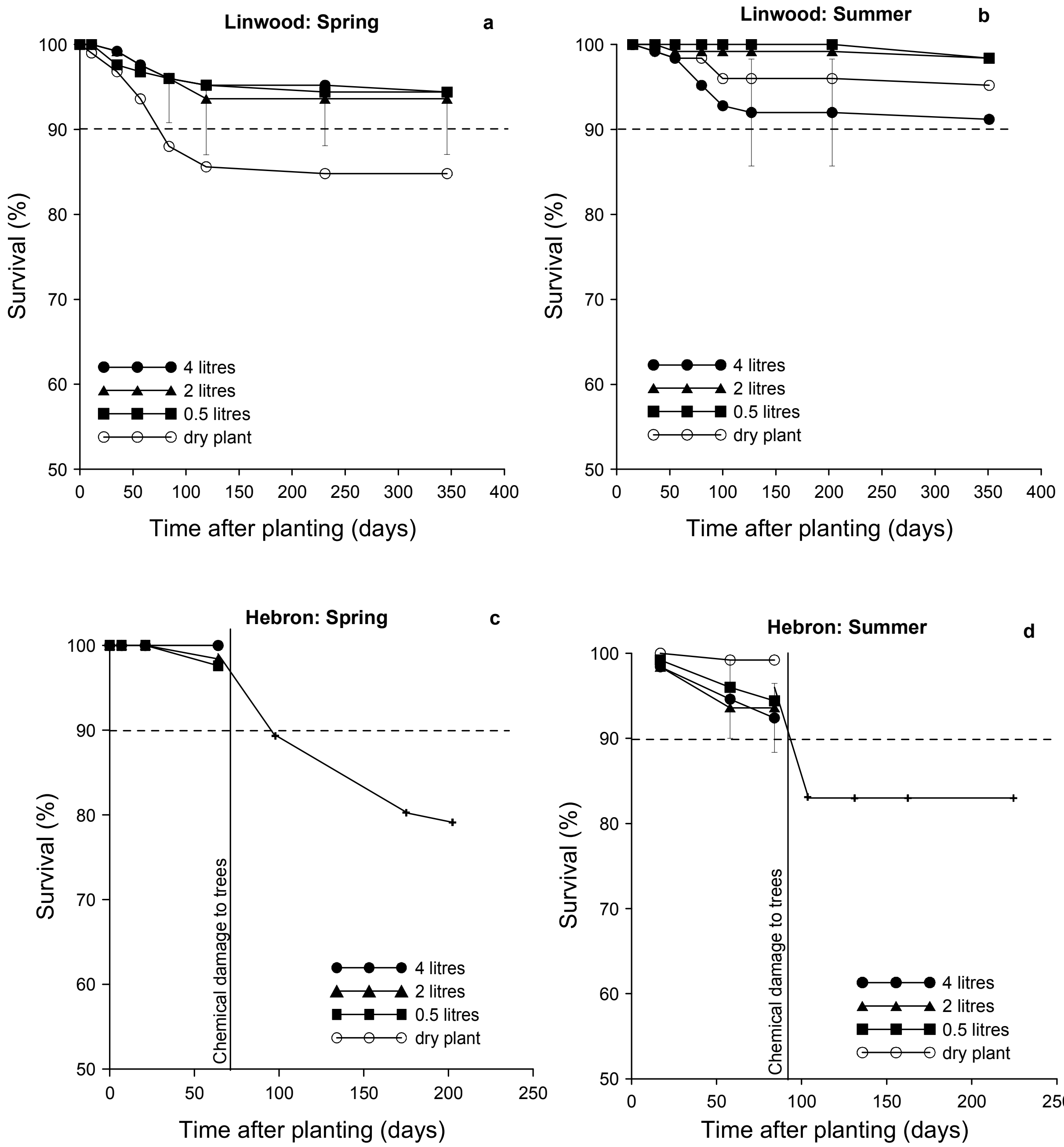


Figure 2.1. Survival of *P. patula* seedlings in response to the of application water (0, 0.5 ℓ, 2 ℓ, 4 ℓ) at planting in four trials established during spring and summer in the KwaZulu-Natal Midlands (Linwood) and Mpumalanga (Hebron). The solid single line following chemical damage at the Hebron trials indicates an average of survival in all treatments. Bars indicate the least significant difference between treatments. The dotted line at 90% represents commercially acceptable survival at 90 days after planting.

Table 2.5. Temperature and rainfall the week before (7 d before) and two weeks after (0-7/14 d) planting at four *P. patula* water planting trials established in spring and summer in the KwaZulu-Natal Midlands (Linwood) and Mpumalanga (Hebron).

Trial	Temperature (˚C)			Rainfall (mm)		
	No. days > 24˚C	Average maximum				
	0-14 d	0-7 d	7-14 d	7 d before[#]	0-7 d	7-14 d
Linwood: spring	10	28.0	25.5	0	6	16
Linwood: summer	12	25.4	28.3		6	56
Hebron: spring	9	18.1	22.8	0	45	7
Hebron: summer	14	26.5	26.7	65	144	63

[#] Shaded cells indicate that data were not obtained.

the dry planted treatment. It is also possible that the slightly larger, more robust seedlings planted during summer were better able to tolerate the hot and dry conditions. Seedling quality at the time of planting has been positively related to survival (Morris, 1994; Généré and Garriou, 1999; Bayley and Kietzka, 1997; Mitchell *et al.*, 2005b). Morris (1994) found that when planting at the end of the summer season (April to May) in Swaziland on sandy, clay loam soils, acceptable survival of *P. patula* could be achieved by the planting of good quality seedlings without the addition of water.

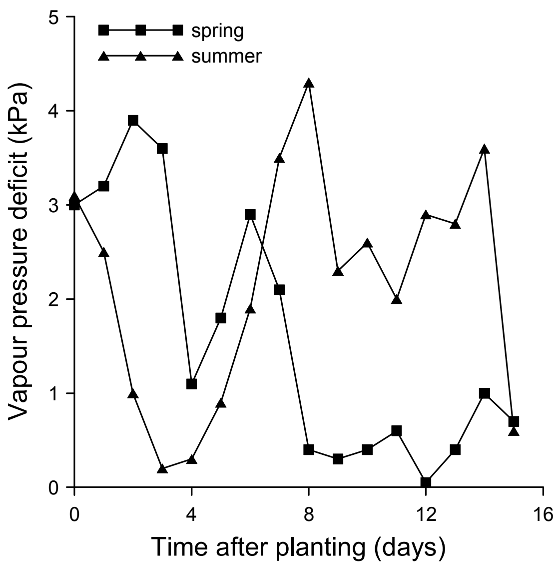


Figure 2.2. Changes in the vapour pressure deficit (kPa) associated with daily maximum temperatures at the Linwood trial planted in spring (October) and summer (February).

Initial survival was good at Hebron (spring and summer), with an average of 98.5% survival at 60 days for the spring and summer planted trials (Table 2.2 and Figure 2.1c-d). No significant differences in survival were detected in the spring trial, whereas survival of the dry planted treatment was significantly better than that of the three treatments planted with water in the summer trial (Table 2.2 and Figure 2.1c-d). There were three cool (air temperatures below 10˚C) days, and 45 mm of rainfall, during the seven days following the planting of the Hebron (spring) trial, with higher air temperatures occurring only seven to 14 days after planting (Table 2.5). The 14 days following the planting of the Hebron (summer) trial were all very hot, however, high rainfall, typical for this region during summer, occurred and initial good survival is likely related to the high soil moisture availability (Table 2.5). Excessive herbicide induced seedling mortality occurred at both trials (at 60 and 90 days) after routine weed control operations, and as such these trials had to be terminated.

2.3.2 Pit soil moisture

A lateral gradient in surface soil moisture content was recorded within all treatments at all four trials (data shown for Linwood only; Figure 2.3 and Figure 2.4). In comparison to the dry plant, the use of 0.5 litres increased soil moisture in the area immediately surrounding the seedling (0 - 0.05 m), whereas 4 litres increased soil moisture laterally throughout the pit (Figure 2.3 and Figure 2.4). Low intensity rainfall events (< 15-20 mm) increased the soil moisture contents recorded for all treatments; however, this increase remained relative to the initial values (Figure 2.3 and Figure 2.4). After more intense rainfall events (> 40-60 mm), especially at the Hebron site, soil moisture contents between the treatments equalized. This extended period of soil moisture differences between treatments, even after low intensity rainfall events, was unexpected. It may reflect either the effect of water during planting on pit soil bulk density or hydrophobicity, where watering at planting may improve water infiltration at the following precipitation event (Bassett, 2008).

Although the soil water retention characteristics at Linwood were not determined, it is possible to get an indication of changes in plant available soil water from studies conducted on similar soil types at low bulk densities (similar to that of disturbed pit soil). The permanent wilting point (which is generally taken to be a soil matric potential of -1.5 MPa) for clay textured soils at low bulk densities will occur when the volumetric soil moisture content (m^3 m^{-3}) drops below 0.28 (or 28%), (Smith *et al.*, 2001). Field capacity (generally accepted to be the soil moisture content at a matric potential of -10 KPa) will be attained at a volumetric soil moisture content of about 0.39 m^3 m^{-3} (or 39%) (Smith *et al.*, 2001). When the seedlings were planted at the Linwood (spring) trial, the soil moisture in

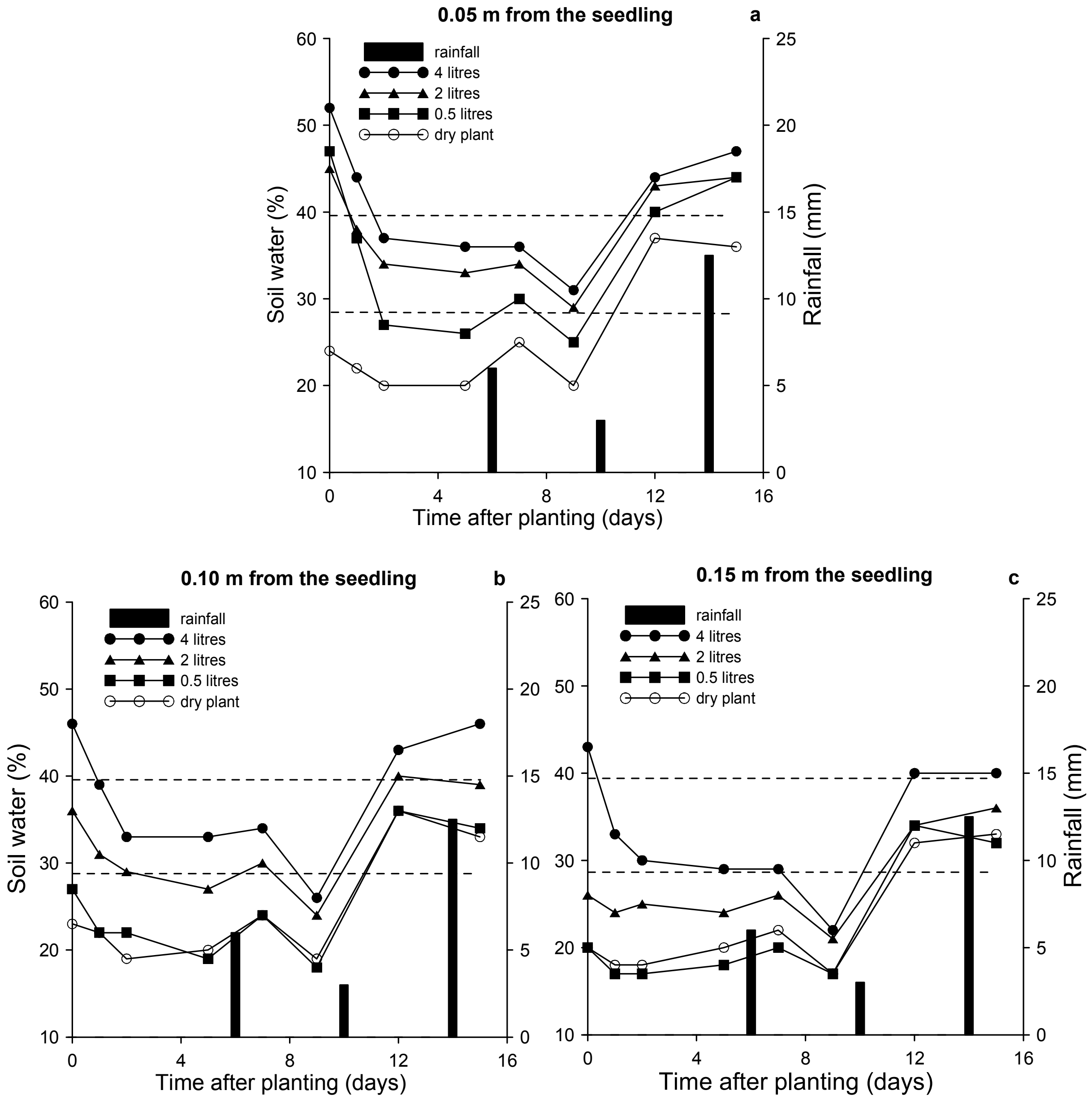


Figure 2.3. Changes in volumetric soil water content (%) of the top 0.06 m of pit soil as measured with the Theta Probe at a distance of a) 0.05 m b) 0.10 m and c) 0.15 m away from the seedling in the Linwood (spring) trial. Bars represent rainfall during the first two weeks following planting. Dotted lines at 39% and 28% soil water represent estimated soil water content at field capacity and wilting point.

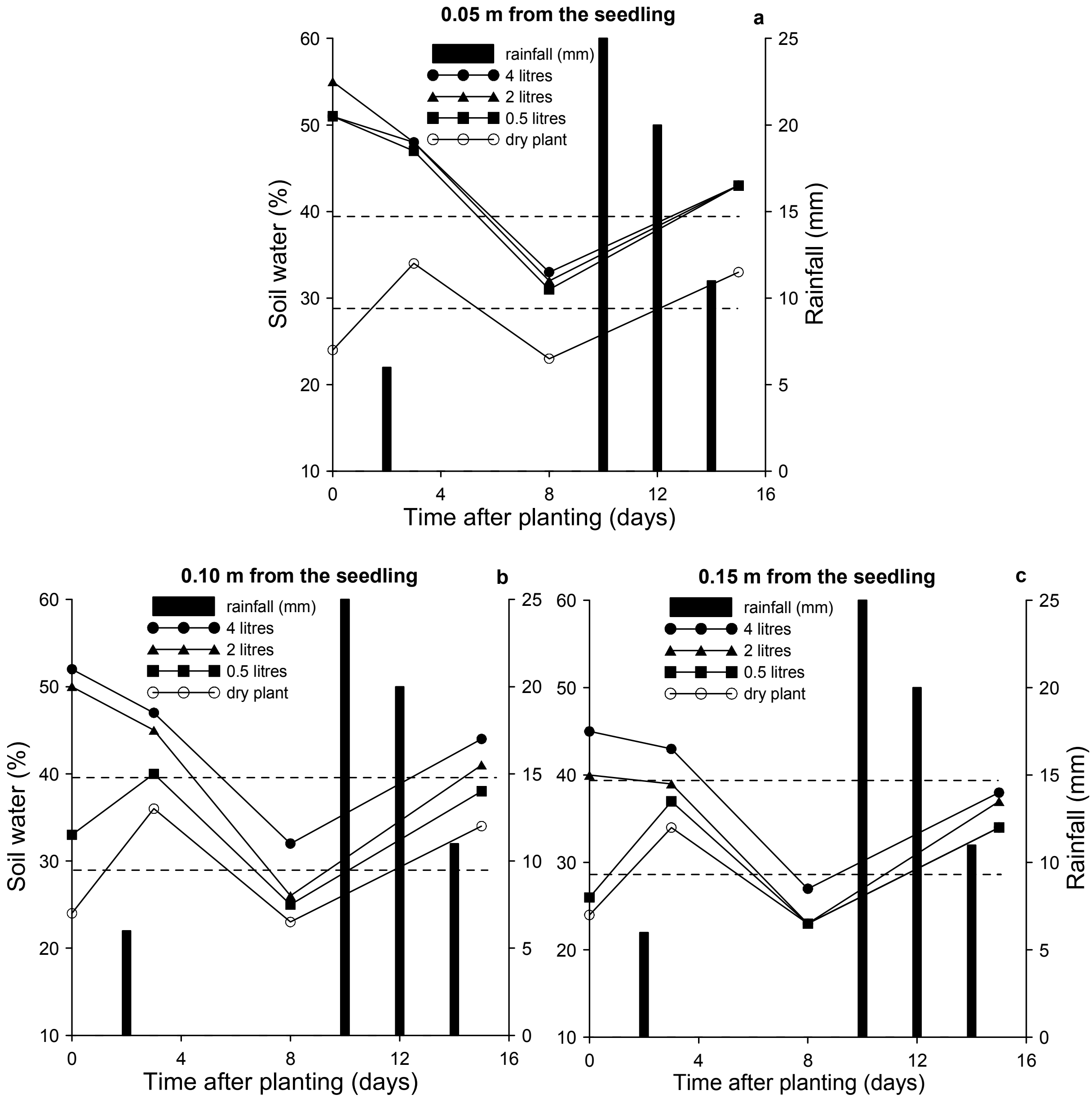


Figure 2.4. Changes in volumetric soil water content (%) of the top 0.06 m of pit soil as measured with the Theta Probe at a distance of a) 0.05 m b) 0.10 m and c) 0.15 m away from the seedling in the Linwood (summer) trial. Bars represent rainfall during the first two weeks following planting. Dotted lines at 39% and 28% soil water represent estimated soil water content at field capacity and wilting point.

the dry pits was near to wilting point for the first nine days after planting (20 to 25%) (Figure 2.3). While soil moisture was near to wilting point during the Linwood (summer) trial, rainfall two days after planting, and during the second week, increased soil moisture to above wilting point. At both planting dates application of only 0.5 litres of water to the soil immediately surrounding the seedling was sufficient to increase soil moisture in the zone around the seedling (0 to 0.05 m) to above wilting point. In addition, this increase in soil moisture persisted for up to two weeks after planting (Figure 2.3 and Figure 2.4).

At the Linwood (spring) trial, survival of the seedlings in the dry planted treatment diverged from the water planted treatments from approximately 30 days after planting, but was only significant at 90 days (Figure 2.1a). This delayed response in survival to a stress experienced from planting may highlight some of the difficulties associated with identifying the point at which pine seedlings die. It seems unlikely that low soil moisture at the time of planting in the dry plant treatment may have been the direct cause of mortality 60 days to 90 days later and therefore the cause and timing of mortality remains unknown. Application of 0.5 litres to the seedlings at the spring trial increased survival to a level equivalent to that where 2 or 4 litres of water was used, yet only increased soil moisture in the area immediately surrounding the seedling. This could reflect that; 1) water (either 0.5 litres, 2 litres or 4 litres) increased soil moisture in the pit for only a short period and was therefore only available to the seedlings in the region immediately surrounding the root plug (and any soil water beyond this zone was unavailable to the seedlings and evaporated by the time the roots had penetrated that far), and (or) 2) the use of water increased root to soil contact at planting, thus improving any subsequent root and soil water interaction.

2.3.3 Soil and air temperature

No differences were detected in pit soil temperature between treatments when measured at the Linwood (summer) trial (data not shown). This may be due to the variability in temperature measurements within treatments as well the small sample size used (n=4 measurements per treatment). The variability in soil temperature measurements within treatments may be a function of the measuring equipment and (or) micro-environmental differences between different pits where factors such as organic matter, soil friability, soil moisture and position of the pit in the landscape may affect the temperature of the soil to a larger extent than the quantity of water that has been artificially added to the soil. Average maximum pit soil temperatures ranged between 18 and 25˚C and minimum temperatures were around 18˚C during the first two weeks after planting (Figure 2.5). Maximum air temperature at 0.10 m in the zone of the shoots at the summer planted Linwood trial, was 3-4˚C higher than those measured at 1.3 m and reached 35˚C or higher on four occasions during the first week after planting (Figure 2.5).

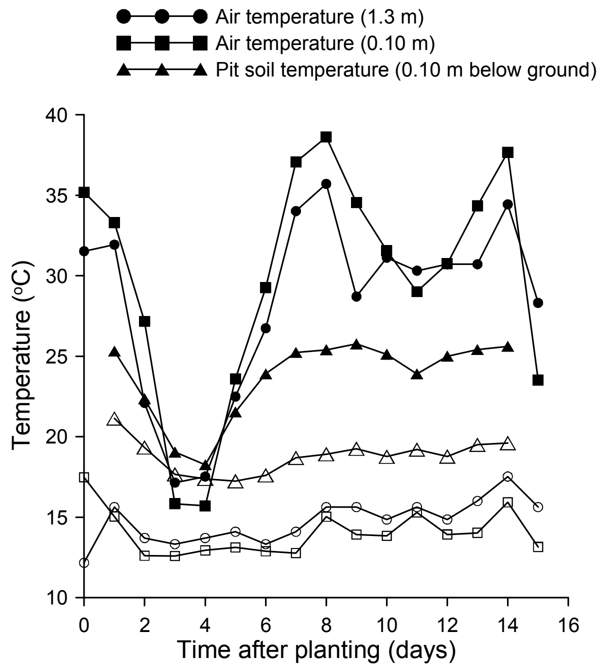


Figure 2.5. Measurements of pit soil temperature (0.10 m below ground) and air temperature (0.10 m; 1.3 m) made at the Linwood trial planted in summer. Closed symbols indicate maximum values and open symbols indicate minimum values.

While no effect of watering on pit soil temperature in the root zone was recorded, data provide an indication of the relationship between ambient air temperature and that of pit soil during the peak summer period. The production of primary lateral roots and new root tips by *Pinus* species is strongly influenced by root zone temperature, water availability and their interaction (Nambiar *et al*., 1979; Brissette and Chambers, 1992; Sword, 1996). The negative effect of water stress on new root growth for *P. palustris* has been found to increase at higher root-zone temperatures (>20˚C), (Sword, 1996). Carlson *et al*. (2004) found that daily exposure of *P. patula* seedlings to soil temperatures above 24˚C, for at least one hour over a period of 10 days, significantly reduced the development of new roots. However, despite the potentially negative impacts of high temperatures on seedling root growth, these data indicate that provided sufficient water is available, high temperature stress is not a primary determinant of mortality in *P. patula*.

The results obtained in these four trials are consistent with several other local studies where planting with water has been shown to increase survival when planting during early spring or late autumn (Morris, 1994; Rolando *et al*., 2006). Besides these studies, however, little literature could be found on the practice of planting with water in forestry worldwide (Evans, 1992), making it difficult to contextualize these results with that of international studies. This is contrary to the agricultural sector where …"the application of water to the soil surrounding the roots of plants immediately after planting is common practice" (McKee, 1981; Cox, 1984). According to McKee (1981) re-establishment of vegetable seedlings is improved by; 1) making water readily available to the root system, 2) reducing water loss to the soil from the roots should the soil be dry at the time of planting and 3) by improving root to soil contact. Cox (1984) investigated the combined effects of root plug moisture content at planting and watering immediately after planting on survival and growth of lettuce and leeks. While watering at planting (with no further irrigation) was found to improve root growth during the first week after planting, it was unable to keep the seedlings supplied with water for more than a few days following planting during summer (Cox, 1984). Cox (1984) emphasized the importance of new root growth to subsequent seedling survival, and yield.

2.4 CONCLUSION

These trials were implemented during periods within the commercial pine planting season when either sporadic rainfall and/or high air temperatures were likely to occur. Measurements of rainfall, air temperature and changes in pit soil moisture (both spatially and temporally) at all trials, as well as intensive measurements of pit soil temperature at one trial were carried out to increase the understanding with which the data could be interpreted. While additional measurements provided some insight into the micro-environment in response to planting treatment, it was not possible to determine the actual cause of seedling mortality. More datasets combined with measures of seedling physiology following planting may increase our understanding in future trials. The results from the treatments and conditions that prevailed in these trials have indicated that when planting *P. patula* seedlings:

- Planting with water has the potential to increase survival when soil water availability is low and rainfall sporadic (but cannot reduce mortality during prolonged drought).
- The amount of water used in the planting operation, on soils similar to that tested in this study, is not critical and either 1 or 2 litres is sufficient, provided some is applied into the planting hole prior to planting. The method of application of the water to the seedling and the effect of this on survival will need further investigation.
- Seedling quality may play an important role in the requirement for water during planting and may require further investigation.
- Planting depth is affected by individual planters as well as planting method. The effect of planting depth on survival has not been investigated locally.

CHAPTER 3

WATER VERSUS DRY PLANTING: A SYNTHESIS OF THREE MONTHS SURVIVAL DATA FOR 58 *PINUS PATULA* RESEARCH TRIALS

ABSTRACT

Despite the implementation of many trials, there is no consensus as to whether planting *Pinus patula* seedlings with water increases survival over those planted without water in the summer rainfall region of South Africa. This is partly a function of the isolation of research in time, as well as the site specific nature of the trials, each reflecting the environmental and climatic conditions at implementation. To address this, a dataset of 58 trials incorporating a dry planted and water planted treatment was evaluated to determine whether trends in three months survival existed. The trials incorporated into the dataset were all planted to *P. patula* between 1990 and 2005 in the summer rainfall region of southern Africa. Data pertaining to the climate, local weather, physiography and site management at each trial were included. Summary statistics, linear correlation and multiple regression were used to determine if site-associated variables were related to survival in the dry planted and water planted treatments. The analyses indicated survival was lowest during the summer months, regardless of treatment. Planting with water was most likely to increase survival when used during spring, autumn and winter planting. Linear correlation and multiple regression did not highlight any significant relationships between site-associated variables and survival in the dry planted and water planted treatments.

3.1 INTRODUCTION

Since the success of regeneration is frequently measured in terms of survival it is important to identify factors that can minimise post-planting stress and mortality. In South Africa, post-planting mortality of *Pinus patula* frequently exceeds the commercially acceptable level of 10% (Bayley and Keitzka, 1997; Crous, 2005; Rolando and Little, 2005). Mortality following planting has been associated with one, or a combination, of the following factors: heat and drought stress; pests and diseases, harvesting residues and seedling quality (Morris, 1990; Bayley and Kietska, 1997; Allan and Higgs, 2000; Rolando and Allan, 2004; Crous, 2005; Rolando and Little, 2005). Post-planting mortality also may be exacerbated by poor nursery to field transport, incorrect planting methods as well as extreme weather conditions following planting. Some of these factors are management related and can be controlled, such as the treatment of harvest residues, planting method, seedling quality and nursery to field transport. Other factors, such as post-planting climatic conditions and outbreaks of pests and diseases are beyond direct control. It is important that research is focused on factors that can be controlled, where management decisions are likely to have a direct impact on survival. This is probably the most important way to minimize mortality and optimize regeneration success in an economically sound manner.

The planting season for *P. patula* in the summer rainfall region of South Africa is predominantly between September and March, when most of the annual rainfall occurs. However, planting during this period is often restricted by low soil water availability and unpredictable rainfall (Zwolinski, 1997; Viero *et al*., 2000; Rolando *et al*., 2006). In addition, the large areas that require re-planting often forces foresters to plant during these seasonal, unfavourable climatic periods to ensure the completion of their scheduled planting programmes. Application of water to the seedling at planting has been used in an attempt to reduce water stress and buffer against potentially extreme post-planting weather conditions (Morris, 1994; Nelson, 1995; Viero *et al*., 2000). However, the primary role of the water, as well as its success in improving regeneration, has never been critically assessed and the economic value of this practice, in South Africa, is questionable.

There are a number of factors that complicate an assessment of the viability of planting with water. Firstly, the use of or method of application of the water during the planting operation varies widely between regions and commercial timber companies. Some companies have a "no water" policy, others always plant with water, while some apply water only when planting conditions are considered poor (i.e. hot weather, dry conditions). Between different companies there is also variation in the quantity of water used as well as the method of application. In South Africa, seedlings are manually planted into prepared planting pits (an area of soil manually loosened with a hoe or mattock). During the planting operation a small planting hole is made in the pit with a hand-held trowel and the seedling is placed into the hole before closing to secure the root plug. When used, water may be poured into the planting hole before placing the seedling (known as a "puddle plant"), poured around the seedling after planting (known as a "drench'), or even both, often depending on the quantity of water used. In terms of quantity, the range is generally one to two litres, although up to five litres is used (ICFR, 1995; 1996a, 1996b; Atkinson and Govender, 1997; Viero *et al*., 2000).

The application of water when planting *P. patula* seedlings does not always improve survival (Atkinson and Govender, 1997; ICFR and Mondi Forests, 1997; Allan *et al*., 2000; Rolando and Little, 2004; Crous, 2005). More often, there is acceptable or unacceptable survival across a trial. This raises questions as to the role of water when planting pines. Is the function of the water to provide root to soil contact and improve water availability to the seedling for the weeks following planting? Does the response to planting with water vary seasonally? To improve our understanding regarding these questions a dataset of 58 trials, incorporating a dry planted and water planted treatment, was compiled to assess trends in survival. The dataset included trials planted throughout the year in the summer rainfall region of South Africa (Mpumalanga and KwaZulu-Natal) and Swaziland, between the years 1990 and 2005 (Table 3.1). Since no similar retrospective studies aimed at assessing the merits of planting with water for *P. patula* could be found in the literature, this assessment had the potential to be important to the South African forestry industry.

3.2 MATERIALS AND METHODS

3.2.1 Description of trial sites and data collection

Only trials planted to *P. patula* that contained both a dry planted (no water applied during the planting operation) and a water planted (water applied to the planting pit during the planting operation) treatment were considered for the dataset. Details of the original trials from which the data were extracted, as well as information pertaining to the area and site characteristics of each trial, are shown in Table 3.1.

Table 3.1. Location and physiographic details of trials used to assess three months survival of *P. patula* seedlings that were either dry planted or water planted. The abbreviations KZN, SWZ and MPU have been used for the areas KwaZulu-Natal, Swaziland and Mpumalanga (southern Africa). References refer to the published results from each trial.

Trial No.	Locality	Plantation	MAP (mm)	MAT (˚C)	Alt (m asl)	Reference
1-2	KZN	Linwood	930	15.7	1300	Rolando and Little (2004)
3	KZN	Linwood	825	16.3	1180	Rolando and Little (2004)
4-6	SWZ	Usutu	1000	16.1	1470	Allan *et al*. (2000)
7-12	SWZ	Usutu	1040	15.2	1530	Allan and Higgs (2000)
13-14	MPU	Hendriksdal	1100	16.5	1300	Atkinson and Govender (1997)
15-18	MPU	Blyfstaanhoogte	1050	14.5	1800	Atkinson and Govender (1997)
19-26	MPU	Dorsbult	760	14.0	1600	ICFR and Mondi Forests (1997)
27-34	MPU	New Scotland	854	14.6	1650	ICFR and Mondi Forests (1997)
35-37	MPU	Hendriksdal	1180	16.2	1500	Allan *et al*. (2000)
38	MPU	Driekop	1233	15.9	1440	Rolando and Little (2004)
39-40	MPU	Hebron	850	14.6	1650	Rolando and Little (2004)
41-42	MPU	Hlelo	817	16.9	1300	Rolando *et al*. (2006)
43; 48	MPU	Mamre	982	14.4	1768	Crous (2005)
44	MPU	Uitkyk	841	14.0	1859	Crous (2005)
45; 54	MPU	Helvetia	770	14.8	1650	Crous (2005)
46	MPU	Sabey	1178	17.4	1178	Crous (2005)
47	MPU	Kalmoesfontein	870	15.6	1725	Crous (2005)
52	MPU	Kalmoesfontein	870	16.2	1570	Crous (2005)
55	MPU	Kalmoesfontein	870	16.7	1439	Crous (2005)
49	MPU	Nooitgedacht	950	17.8	1140	Crous (2005)
50	MPU	Nooitgedacht	950	15.1	1600	Crous (2005)
51	MPU	Nooitgedacht	950	14.9	1600	Crous (2005)
53	MPU	Nooitgedacht	950	17.8	1140	Crous (2005)
56	MPU	Nooitgedacht	950	14.5	1870	Crous (2005)
57	MPU	Grootgeluk	732	16.9	1274	Crous (2005)
58	MPU	Ndubazi	817	15.3	1494	Crous (2005)

For each trial, three months survival data were analysed using an ANOVA appropriate to the original trial design (Steel and Torrie, 1980). Survival of the dry planted and water planted treatments were extracted and incorporated into the database. Information related to site management, quantity and method of application of water, climate (and weather), physiography and planting date were also included for each trial (Table 3.2). For some trials it was not possible to obtain all the relevant information, especially the weather data specific to the site during the month of the planting operation.

Table 3.2. Description of the variables assessed for each trial and used in the assessment of factors affecting the three months survival of *P. patula* seedlings that were either dry planted or water planted.

Variate assessed	Abbreviation used in text	Description of variate
		Response variable
Survival	*survival*	Percentage survival of trees in the dry planted or water planted treatment at each planting event. Data were arcsin transformed prior to analyses.
		Explanatory variables
Mean annual temperature	*MAT*	Mean annual temperature for the site on which the trial was planted. Data were obtained either from the Forest Productivity Toolbox (Version 1.3)[#] or from the nearest forestry office to the site.
Mean annual precipitation	*MAP*	Mean annual precipitation for the site on which the trial was planted. Data were obtained either from the Forest Productivity Toolbox (Version 1.3)[#] or from the nearest forestry office to the site.
Altitude	*alt*	The altitude (m asl.) of the site was obtained either from the Forest Productivity Toolbox (Version 1.3) or from a 1:10 000 / 1:50 000 map obtained from the relevant forestry office.
%Clay	*clay*	The percentage clay in the soil at the site. The data were grouped as follows: 1: 20-35% clay 2: 36-55% clay 3: >55% clay None of the sites had less than 20% clay.
Season	*season*	The season of planting: spring (September to November) summer (December to February) autumn (March to May) winter (June to August)
Method	*method*	Data were scored, according to the method of application of the water, as follows: 0 = water poured as a drench around the seedling after planting 1 = water poured into the planting hole during planting of seedling
Air temperature	*temp_avmax* *temp_maxt>30*	During the 14 days following planting, the air temperature was recorded as the average of the daily maximum air temperature (˚C) (*temp_avmax*), as well as the number of occasions the daily maximum air temperature was greater than or equal to 30 ˚C (*temp_maxt>30)*. Data were log transformed prior to analyses.
Rainfall	*rain_tot* *rain_noday*	The total amount of rainfall during the month (30 days) following planting (*rain_tot*) and the number of days over which this rainfall occurred (*rain_noday*). Data were log transformed prior to analyses.
Quantity	*qnty*	The quantity of water (litres) applied to the seedling during the planting operation.
Harvest residue management	*slash*	The management of the residue prior to planting. 0 = clear (harvest residue removed from the treatment plot) 1 =broadcast (the harvest residue was broadcast across the treatment plot)

[#] Forest Productivity Toolbox (Version 1.3) is a software forestry information package developed by the Institute for Commercial Forestry Research, Pietermaritzburg, South Africa.

3.2.2 Exploration of data and description of analyses

Commercially, blanking (replacing of dead trees) is only carried out if the survival falls below 90% during the first three months after planting (Morris, 1995). For this study, it was assumed that most mortality in response to planting with or without water would have occurred during the first three months. While further mortality between three months and one year after planting occurs (Rolando and Little, 2005), it is unlikely to be related to the application of water during the planting operation.

To understand the trends in three months survival in the water planted and dry planted treatments, summary statistics and simple linear regression were used. Scatter plots, linear correlation and multiple linear regression were used to determine if any of the site management, physiographic or climatic variables (explanatory variables) could be related to three months survival (response variable) (McConway *et al.*, 1999). All analyses were carried out using the statistical package Genstat® for Windows™ Version 9 (Payne *et al.*, 2006). Survival data were arcsin transformed prior to analyses. Where necessary physiographic and climatic data were transformed to meet the assumptions of the statistical tests used (Steel and Torrie, 1980) (Table 3.2).

3.3 RESULTS

The three months survival of the dry planted and water planted treatments was similar, 80.3 ± 22% and 83.7 ± 18% (Table 3.3). The survival of both were negatively skewed, with over 80% survival for most planting events (Figure 3.1). A paired two-tailed *t*-test, conducted on the arcsin transformed survival data, indicated no significant difference between the two treatments (t=1.55, df=57, p=0.127). However, plotting the survival of the water planted trees against those that were dry planted indicated that planting with water generally increased survival, particularly when survival was below 90% (Table 3.4; Figure 3.2a). This effect was most pronounced in autumn and winter with no affect of water when planting during summer, as indicated by the regression analysis with seasons included as a grouping factor (Table 3.4; Figure 3.2b). The robustness of this regression analysis would be improved if more data were available for autumn (n=13) and winter (n=9). Regardless of whether seedlings were dry planted or water planted, survival was generally the best in autumn, with the poorest survival occurring during summer, where there was an average survival of 75% (Figure 3.3). The difference in the average survival of dry planted and water planted seedlings was greatest in winter, with the highest variability associated with survival of winter dry planted seedlings (Figure 3.3).

Commercially, blanking is usually carried out when survival within a three months period falls below 90%. Since blanking is a costly operation, it is better to ensure that more than 90% of the original plants survive at the three months cut-off period. In this dataset, the application of water increased the survival from below 90% to above 90% in only 6 of the

Table 3.3. Summary statistics for the quantitative response and explanatory variables used in the assessment of survival of *P. patula* seedlings that were either dry planted or water planted.

Variate	n	Mean (±sd)	Range	Coefficient of Variation (%)
Water planted trees: *survival* (%)	58	83.7 ±18	17.6-100	22.4#
Dry planted trees: *survival* (%)	58	80.3 ± 22	12.1-100	27.4#
Mean annual temperature: *MAT* (˚C)	58	15.4 ±1.0	14.0-17.8	7.2
Mean annual precipitation: *MAP* (mm)	58	950.0 ±163	732-1570	17.1
Altitude (m asl): *alt*	58	1533.0 ±189	1137-1865	12.0
Air temperature: *temp_avmax* (˚C)	29	23.4 ±4.6	15.3-29.0	19.7
Air temperature (days): *temp_maxt>30* (˚C)	32	4.4 ±5.2	0-17	119.0#
Rainfall: *rain_tot* (mm)	32	96.3 ±89.2	0-297	92.7#
Rainfall: *rain_noday* (days)	32	8.0 ±5.2	0-19	70.0#
Quantity: *qnty* (litres)	58	2.1 ± 0.7	1.0-4.0	37.6

data were transformed for further analyses. See Table 3.2 for details.

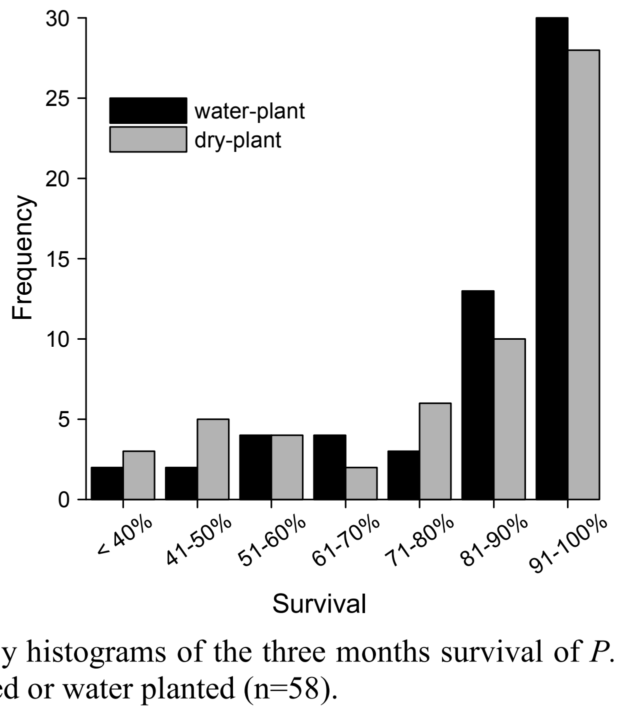


Figure 3.1. Frequency histograms of the three months survival of *P. patula* seedlings that were either dry planted or water planted (n=58).

Table 3.4. Summary of the linear regression analyses of survival (n=58; data arcsin transformed) for the water planted treatment expressed as a function of that in the dry planted treatment (All data). The data were separated into seasons to test for the effect of season on the regression (Seasonal data). See Figure 3.2a (All data) and Figure 3.2b (Seasonal data).

Source of variation	**All data**		**Seasonal data**	
	df	**Mean square**	**df**	**Mean square**
Regression	1	7750.9**	7	1359.8**
Residual	56	66.1	50	38.6
Total	57	200.9	57	200.9
Accumulated analysis of variance				
+Treat			1	7750.6**
+Season			3	213.1*
+Treat.Season			3	376.3**
Residual			50	38.6
Total			57	

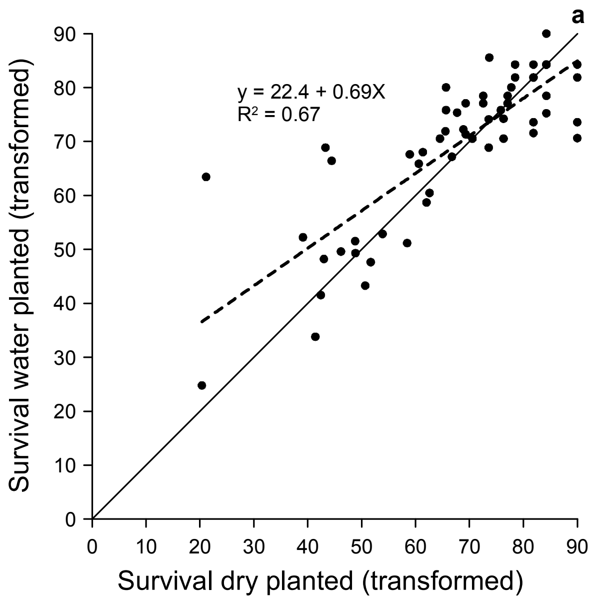
* significance at p<0.05, ** significance at p<0.01

58 trials (10%), with an additional three events where water planting increased the average survival by 35% or more. Based on the available data, it is possible to estimate a theoretical probability of survival greater than 90% for each season as affected by the application of water (where the probability that an event will occur is calculated as the relative frequency of the event) (Steel and Torrie, 1980). These data indicate that during spring the probability of survival greater than 90% was improved with water planting (Table 3.5). During summer, when survival was generally poorer (Figure 3.3), the probability of survival greater than 90% was low (p<0.37), regardless of whether water was used in the operation or not. During winter, the probability of survival greater than 90% was 0.78 regardless of the planting method (Table 3.5). The smaller sample of winter plantings may be skewing the data for this season and this interpretation may not be sound.

Table 3.5. Probability of survival of 90% and greater for the dry planted and water planted treatments across seasons.

Treatment	**Spring**	**Summer**	**Autumn**	**Winter**
	n=13	n=19	n=17	n=9
Dry plant	0.38	0.32	0.59	0.78
Water plant	0.61	0.37	0.59	0.78

Linear correlation, scatter plots, and multiple regression of the explanatory and response variables did not highlight any potential predictor variables that could account for three months survival in the dry planted and water planted treatments.



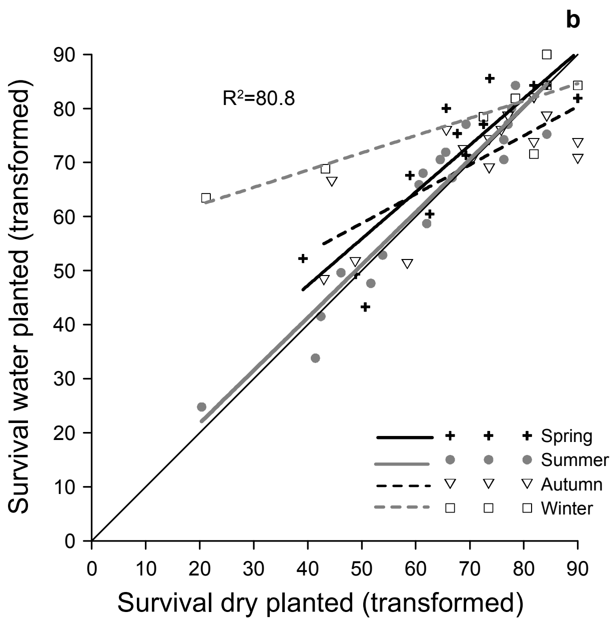


Figure 3.2. (a) A comparison of the three months survival (n=58; data arcsin transformed) of *P. patula* seedlings that were either dry planted or water planted. The dashed line represents the regression equation. The solid line through the origin indicates equality. Points above the solid line represent cases where planting with water increased survival. (b) The same data have been separated according to season of planting (Table 3.4).

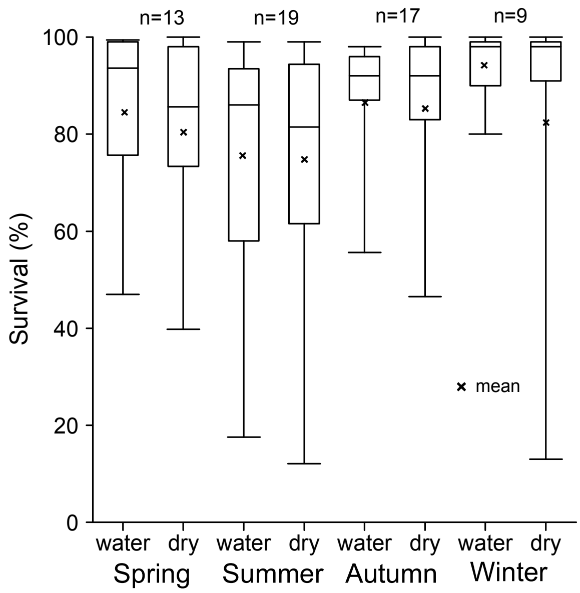


Figure 3.3. Box and whisker plots for three months survival of dry planted and water planted *P. patula* seedlings across seasons. The caps at the end of each box indicate the extreme values (minimum and maximum), the box is defined by the lower and upper quartiles, and the line in the centre of the box is the median. The mean has been also indicated on the plots.

3.4 DISCUSSION

These data have indicated that planting *P. patula* seedlings with water does not always increase survival, highlighting a current lack of knowledge of the water-relations of *P. patula* seedlings during regeneration. The likelihood of a positive response in survival to the use of water was affected by the season of planting, with a greater chance of a positive response when planting during early spring or late autumn. Rolando *et al.* (2006) and Rolando and Little (2007) also showed water planting had the potential to increase survival only during periods of low soil water availability, particularly during spring. Critical to the management decision of whether to use water during these periods will therefore be the cost of the application of water. An increase in survival in response to the use of water may not exclude a blanking operation within three months. Since both watering and blanking are costly operations, the minimum increase in survival (and yield at felling) required to recover the regeneration costs associated with water planting will therefore need to be determined.

The results show that planting *P. patula* with water during the summer did not increase survival over that dry planted. Heavy rains are common during the summer months and a high soil moisture content is likely to buffer against any dry periods. More importantly, the data indicate that summer planting is less likely to achieve the 90% three month commercial survival target. Morris (1991) and Rolando (2006) also found that survival of *P. patula* seedlings decreased following planting during the summer months and related this to damage from the bark beetle *Hylastes angustatus*. Any benefits from planting with water during the summer period are therefore most likely to be achieved if an insecticide is added to the water. Winter planting is not a common practice in the summer rainfall region of South Africa as high mortality can result from extreme cold temperatures or frost events during winter. However, more data on survival in response to planting with water during autumn and winter, as well as an increase in the understanding of the drought tolerance of *P. patula* seedlings, could show that late season planting, with water, is a feasible option on certain sites.

The lack of any functional relationships between the explanatory and response variables may be due to a number of factors. Firstly, the dataset may be too small to detect differences in response to planting with, or without water, given the variability in both the explanatory and response variables. Secondly, since the weather data surrounding the planting date for some of the planting events was obtained from forestry offices and collected by the local forester, the accuracy of these data may questionable. Thirdly, there may be other, more important explanatory variables, that affect the response (negative or positive) to planting with water, such as, plant quality, method of application of water and soil moisture at the time of planting. It was not possible to test the importance of these variables with this data set, but they should be included in any future trials assessing the viability of the application of water to the seedling at the time of planting.

It is important to highlight that most of the data used in this study were obtained exclusively from research trials where close supervision ensured the practice of good planting. While the data may reflect a true response in survival of *P. patula* seedlings to dry planting or water planting, the nature of the treatment response may differ under commercial conditions. For example, in a commercial operation, the application of water may improve survival over all seasons simply because the planter spends more time tending to the planting hole and planting more carefully.

3.5 CONCLUSION

Most post-hoc, retrospective data collections and analyses suffer from discrepancies reflecting the diverse sources from which the data were obtained. However, this is often the only manner in which responses to treatments can be analysed on a macro-scale. This study did not provide any definite “yes” or “no” to the practice of water planting for *P. patula,* but has shown that the species is potentially more tolerant to drought stress or low soil water availability at planting than previously expected. The range in three months survival across trials included in the study have also indicated that there are many other, possibly more important factors, that affect survival of pines during regeneration. Planting with water may reduce the risk of mortality during certain seasons. However, any management decision to plant with or without water should be made on an economic rationale, highlighting the need to determine the minimum increase in survival (or final yield) required to recover the additional costs of using water in the planting operation.

CHAPTER 4

MEASURING WATER STRESS IN *PINUS PATULA* SEEDLINGS

ABSTRACT

A pot trial was conducted to investigate measures of water stress for *Pinus patula* seedlings as used for commercial forestry in South Africa. The objectives were to determine the efficacy of different equipment for quantifying water stress, and the effect of soil water availability at transplanting on seedling physiology. There were two dry soil treatments differing in terms of seedling root plug moisture at transplanting, dry (DD) and wet (WD), respectively, and three treatments consisting of well watered seedlings planted into wet soil (WWD, WWW and Control). Treatment WWD received no further water after planting while WWW was re-watered when seedlings were water stressed. The Control was maintained at field capacity for the trial period. Seedling physiology (shoot water potential, stomatal conductance and chlorophyll fluorescence) and soil temperature and water content were measured. Shoot water potential and stomatal conductance reflected plant physiological responses to changes in soil water content. Chlorophyll fluorescence measurements were variable and did not reflect treatment effects. A wet root plug at the time of transplanting increased seedling shoot water potential for three days in dry soil. Planting into wet soil increased shoot water potential for the duration of the trial. Soil water content affected soil temperature, with differences exceeding 5˚C recorded on days with air temperatures over 30˚C.

4.1 INTRODUCTION

Pinus patula is native to the misty, mountainous regions of tropical Mexico where annual precipitation varies between 1000 to 2500 mm and average temperatures range from 10 to 18˚C (Dvorak *et al*., 2000). This species is the most widely planted of the softwood species in the summer rainfall region of South Africa (DWAF, 2005/06). Sites considered optimum for *P. patula* exceed 1000 m above sea level, receive more than 850 mm annual rainfall and have a mean annual air temperature of less than 18˚C (Dvorak *et al*., 2000; ICFR, 2005). High post-planting mortality of *P. patula* is common in South Africa, even when planted on optimum sites. Empirical research has indicated that heat and water stress, pests and diseases and the method of harvest residue management may be associated with mortality (Morris, 1990; Bayley and Kietska, 1997; Allan and Higgs, 2000; Rolando and Allan, 2004; Rolando and Little, 2004). In South Africa, climatic conditions during the summer planting season (October to March) can be sub-optimal for seedling growth, even on sites considered suited to *P. patula*. Maximum daily air temperatures can range from over 30˚C to below 10˚C within a few days, and rainfall can be sporadic (Rolando and Little, 2007). Besides one study on the effect of high temperatures on *P. patula* seedling physiology (Carlson *et al*., 2004), no other literature could be found on the ecophysiology of *P. patula* seedlings. To understand the potential effects of high post-planting temperatures and water stress on seedling physiology, and the implications for survival and disease susceptibility, there is a need for more fundamental studies.

Most studies on the mechanisms of drought tolerance and critical limits of water stress in pine seedlings have been conducted on species known to occur naturally in regions with low annual rainfall (< 500 mm), or on temperate species, such as *P. ponderosa*, *P. palustris*, *P. sylvestris*, *P. edulis*, *P. nigra* and *P. taeda* (Kolb and Robberecht, 1996; Rundel and Yoder, 1998). There are limited data available on the ecophysiology of tropical (Mexican) pine species, such as *P. patula*, *P. tecunumanii* and *P. greggii*, despite their economic importance (Rundel and Yoder, 1998). In a study to determine the field performance of several pine species relative to competition control, Capo-Arteaga and Newton (1991) found the tropical Mexican pine species *P. ayacahuite*, *P. montezumae* and *P. hartweggii* to be more sensitive to heat and drought conditions than the drought tolerant pine *P. ponderosa*.

Water planting (application of water into the planting hole at the time of planting) of *P. patula* seedlings has been used commercially in South Africa to reduce post-planting water stress and buffer against potentially harsh weather conditions immediately after planting (Morris, 1994; Allan *et al*., 2000; Oscroft *et al*., 2000; Rolando and Little, 2004). However, the primary role of the water, as well as the potential to increase survival has never been critically assessed. Since the use of water in the planting operation is costly, it is important that the benefits of water planting, both physiological and economic, are quantified.

Since water and heat stress may cause *P. patula* seedling mortality, a quantitative measure of water stress in response to planting treatments during the weeks following planting, may provide insight into measures (treatments) that alleviate water stress. Parameters that can be assessed to determine changes in seedling physiology in response to environmental stress include: biomass partitioning, plant water content, photosynthetic rate, xylem pressure potential, transpiration rate, stomatal conductance, hydraulic conductance, osmotic potential, chlorophyll fluorescence and leaf and stem temperature (for example: Kolb and Robberecht, 1996; Kavanagh and Zaerr, 1997; Rolando and Little, 2003). Factors that limit the number of parameters that can be assessed include manpower, available funding, access to equipment and the accuracy and ease with which the equipment is able to measure the subject. In South Africa, containerised *P. patula* seedlings are generally seven months of age at planting, with a height and root collar diameter of 10 to 25 cm and 0.2 cm, respectively. Often only primary needles (2 to 3 cm in length) are present on the shoot at planting and after planting, average seedling height may be less than 10 cm. Finding equipment to accurately, and non-destructively, measure the physiology of small seedlings with primary needles is difficult. While there are numerous references in the literature to physiological measures of pine seedlings, these are generally applicable to larger, older seedlings, often established in a bare-root nursery system (Kaufmann, 1977; Brissette and Chambers, 1992; Généré and Garriou, 1999; Stanosz *et al.*, 2001). Since there are few references to the measurement of physiological parameters of *P. patula* seedlings, worldwide and in South Africa, the efficacy of different methods in quantifying water stress needs to be assessed. A controlled study (pot trial) incorporating various watering regimes was initiated to determine the efficacy of different types of available research equipment in quantifying water stress in small *P. patula* seedlings as well as to investigate the effect of soil water content at transplanting on seedling physiology.

4.2 MATERIAL AND METHODS

4.2.1 Description of trial and treatments

The trial was carried out at the Institute for Commercial Forestry Research (ICFR) nursery, Pietermaritzburg. The pots (25 cm diameter x 15 cm deep), chosen to reflect the dimensions of standard planting pits prepared for planting, were filled with the equivalent of four litres of a dry, silty clay soil (47% silt, 45% clay and 8% sand). They were sheltered during rainfall events to prevent inadvertent wetting of the soil (and seedlings), as all watering in the trial was controlled. *P. patula* seedlings for the trial were raised in composted pine bark in polystyrene trays with 128 cavities, each with a capacity of 36 ml. The average height and root collar diameter of the seedlings prior to planting was 11.1 ±1.5 cm and 1.8 ±0.2 mm.

Five watering treatments (Table 4.1) were arranged in a randomised complete block of eight replications. The treatments were designed to simulate different levels of soil water availability at the time of planting and immediately thereafter. Each replicate of a particular treatment consisted of a single seedling planted in a pot. Extra seedlings were planted for destructive sampling in WD and WWD. Excepting for the seedlings in DD**,** which did not receive any water on the day before planting, all the other seedlings were watered twice to ensure moist root plugs when planted. The seedlings were planted into the pots in spring (10th September 2004). The trial was terminated 11 days later on the 21st September when water stress was first detected in WWD and sufficient data had been collected to evaluate the efficacy of the equipment being tested.

Table 4.1. Description of treatments used in a pot trial implemented to determine the effect of water availability at and after planting on water stress of *P. patula* seedlings.

No.	Root plug moisture at planting	Water applied at planting	Water applied after planting	Treatment aim	Treatment name
1	Dry	0 litres	0 litres	Dry plant, water stressed seedling, no post-planting rain	DD
2	Wet	0 litres	0 litres	Dry plant, non-stressed seedling, no post-planting rain	WD
3	Wet	1.5 litres	0 litres	Water plant, non-stressed seedling, no post-planting rain	WWD
4	Wet	1.5 litres	1 litre at 9 days	Water plant, non-stressed seedling, post-planting rain	WWW
5	Wet	1.5 litres	1 litre every 1-2 days	Never water stressed	Control

4.2.2 Measurements of environmental conditions

Air temperature (1.5 m above ground) and relative humidity were measured every 15 minutes for the duration of the trial with an Onset Hobo® logger housed in a Stevenson Screen. Vapour pressure deficit (kPa) was derived from measurements of air temperature and relative humidity (Unwin, 1980). Measurements of the soil temperature in the zone of the root plug (0.10 m, below the soil surface) were made with copper-constantan (Type T) thermocouples from the time of planting until trial termination (Campbell Scientific, 1997). One thermocouple was placed at each measurement point in five pots for each treatment, except in treatment DD as it was assumed that pot soil temperature would be similar to treatment WD. Measurements of the volumetric water content (m^3 m^{-3}) of the top 0.06 m of soil in the pots were made with a Delta-T Theta Probe type ML2 (Delta-T Devices Ltd), (Little *et al*., 1996). Using the probe, measurements of soil water content were taken in each pot for all treatments on the day of planting, and at 3, 5, 7, 9 and 11 days after planting.

4.2.3 Seedling measurements

The height (cm), groundline diameter (mm), root and shoot oven dry mass (g) of the seedlings in all treatments were measured on the day of planting and at treatment termination. A sample of 10 seedlings was used to determine the oven dry mass at the time of planting, whereas the seedlings in the pot trial were used for determination of oven dry mass at treatment termination. The oven dry mass of new roots emerging from the root plug, as well as the length of the longest new root, was also determined at termination.

Measurements of stomatal conductance (mmol m^{-2} s^{-1}) were made with a LI-1600 Steady State porometer fitted with a cylindrical chamber recommended for measurement of conifers, with standard operating procedures followed (LICOR, 1984). As the chamber was too small to enclose the entire seedling only the top 2 to 3 cm of each seedling was inserted into the chamber and a stable displayed resistance was taken to indicate that a null-balance had been achieved. Measurements were made on all seedlings at mid-day on the day before planting, and at 1, 3, 5, 7, 9 and 11 days after planting.

Measurements of shoot water potential (MPa) were made using the pressure chamber technique (Scholander *et al.*, 1965). Since the primary needles were too small to measure, measurements were made on the excised stem, and needles (and were therefore destructive). Measurements were made on four seedlings at mid-day on the day before planting and at 1, 3, 7, 9 and 11 days after planting. An assumption that was implicit in the study was that the unharvested seedlings had the same mean shoot water potential as the harvested seedlings.

Chlorophyll fluorescence transients were measured on the primary needles with a portable fluorimeter (Hansatech Plant Efficiency Analyser, PEA) as described by Rolando and Little (2003). All samples were dark adapted for 20 min. prior to fluorescence measurements. Biolyzer 3.0 (Maldonado-Rodriguez, 2002) was used to view the fluorescent data whereby a variety of JIP-test measurements describing a fluorescence induction curve were obtained from the data stored by the PEA (Strasser *et al.*, 2000; Rolando and Little, 2003). Strasser *et al*. (2000) detail the derivation of the multitude of JIP-test parameters, the description of which is beyond the scope of this study. Measurements were made on all seedlings at mid-day on the day before planting and at 1, 3, 5, 7, 9 and 11 days after planting.

4.2.4 Analyses

Although the trial was laid out as a randomised complete block design, the small sample numbers combined with sequential destructive sampling of selected treatments meant that analyses of variance would have been inappropriate. Therefore, treatment means and standard deviations were used to summarise the data. All calculations were carried out using Genstat® for Windows™ Version 9 (Payne *et al.*, 2006).

4.3 RESULTS

4.3.1 Environmental conditions

Although the trial was initiated on a relatively cool day, most of the following days were hot, with air temperatures exceeding 30°C for six out of the 11 days. Mid-day relative humidity was generally low resulting in high vapour pressure deficits (Figure 4.1 and Figure 4.2). All measurements of plant water stress were conducted on clear, sunny days, except on day nine, which was overcast and cool with higher relative humidity (Figure 4.1).

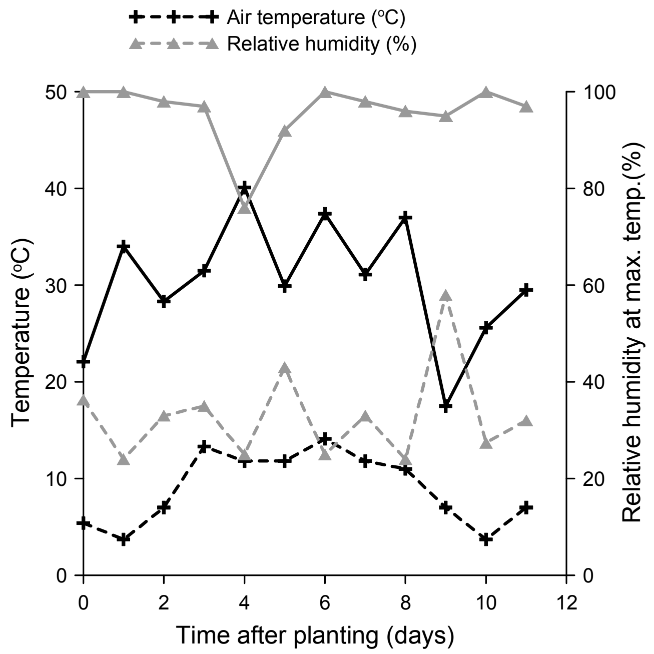


Figure 4.1. Maximum and minimum daily air temperature and relative humidity at maximum daily temperature for the duration of a pot trial implemented to determine the effect of soil water availability at, and after, planting on water stress in *P. patula* seedlings. Solid lines indicate maximum values and dashed lines minimum values.

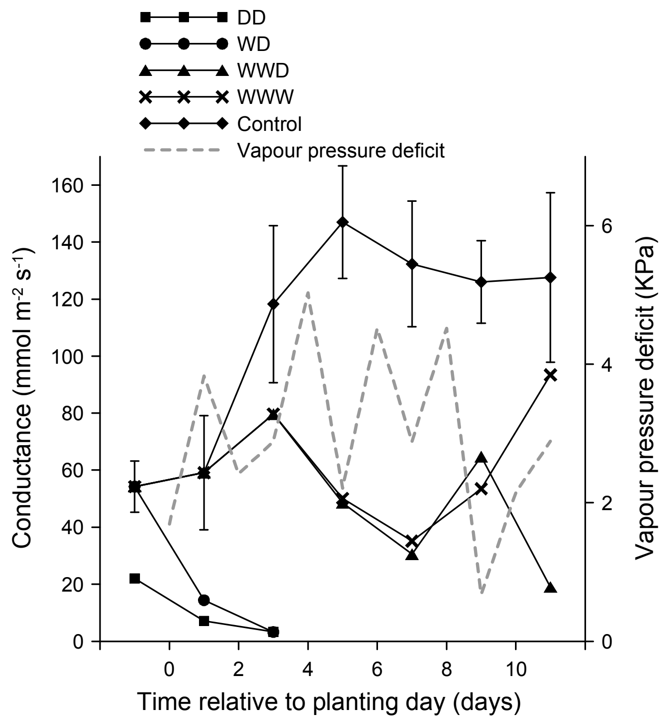


Figure 4.2. Stomatal conductance (mmol m^{-2} s^{-1}) for all five treatments in a pot trial implemented to determine the effect of soil water availability at and after planting on water stress in *P. patula* seedlings. Bars represent the standard errors of the mean at each measurement date. The vapour pressure deficit at maximum daily temperature has been shown.

The water retention characteristics of the soil used in this trial were not determined. However, it is possible to get an indication of plant available soil water from studies conducted on similar soil types. The soil used in this trial was a silty clay, with high organic carbon (10%) and low bulk density (the soil had been disturbed prior to potting). The permanent wilting point occurs at a soil matric potential of -1.5 MPa and Smith *et al.* (2001) estimated that for this type of soil it will occur when the volumetric soil water content ($m^3 m^{-3}$) drops below 0.3 (or 30%). Field capacity (generally accepted to be the soil water content at a matric potential of -10 KPa) will be attained at a volumetric soil water content of about 0.4 m^3 m^{-3} (or 40%) (Smith *et al.*, 2001). Soil water was well above wilting point for the Control for the duration of the trial (Figure 4.3). For WWD and WWW the soil water content dropped to wilting point at seven days after planting. For WD and DD, there was no plant available soil water from the start of the trial.

The treatments affected the daily maximum soil temperatures in the zone of the root plug (Figure 4.4). Initially, soil temperatures were highest in WD (and DD) where the soil was dry (Figure 4.3). Soil temperatures in these pots exceeded soil temperatures of the Control, WWD and WWW treatments by more than 5˚C during the first three days after planting. As the soil in WWD and WWW dried out, maximum daily temperatures started to exceed those of the Control. This reached a maximum on the eighth day after planting when the soil temperature in WWD and WWW was 6˚C higher than that in the Control. Daily minimum soil temperatures of all treatments where similar (Figure 4.4).

4.3.2 Measures of seedling growth and physiology

Average seedling height and groundline diameter after planting were 9.6 ±1.4 cm and 0.25 ±0.03 cm. Treatments DD and WD were terminated three days after trial initiation, with no measurable increase in root and shoot biomass. Similarly, when WWD, WWW and the Control were terminated, there was no detectable increase in average shoot dry mass. However, root mass had increased an average of 0.15 g with new roots emerging from the root plugs (Table 4.2). There was no difference in the mass of new roots produced by the seedlings in WWD, WWW and the Control, although new roots were slightly longer where soil water was not limiting (Control).

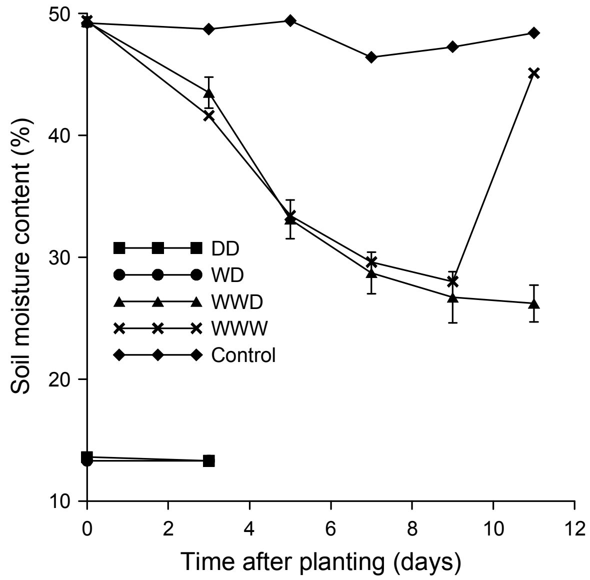


Figure 4.3. Volumetric ($m^3\ m^{-3}$) water content, expressed as a percentage, of soil in pots in a trial implemented to determine the effect of soil water availability at, and after, planting on water stress in *P. patula* seedlings. Bars represent the standard errors of the mean.

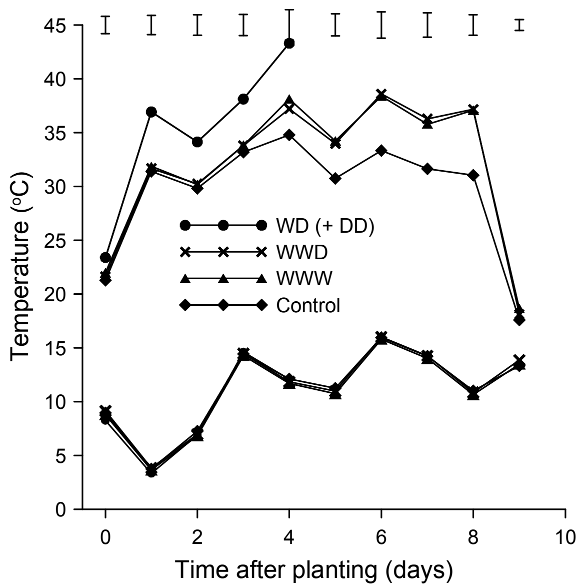


Figure 4.4. Average maximum and minimum daily soil temperature measured for each treatment in the root plug zone (0.10 m below the soil surface) in a trial implemented to determine the effect of soil water availability at and after planting on water stress in *P. patula* seedlings. Bars represent the standard errors of the mean.

Table 4.2. Shoot and root dry mass, before and after planting, for all treatments used in a pot trial implemented to determine the effect of soil water availability at, and after, planting on water stress in *P. patula* seedlings. Values in brackets are the standard deviation of the mean.

Treatment*	Roots in plug (g)	Shoots (g)	New roots (g)	Root:shoot	Length longest new root (cm)
Before planting	0.92 (±0.06)	1.10 (±0.15)	-	0.83	-
DD (3)	1.05 (±0.07)	1.10 (±0.10)	0	0.98	0
WD (3)	1.03 (±0.10)	1.00 (±0.06)	0	1.01	0
WWD (11)	1.16 (±0.20)	1.30 (±0.20)	0.023 (±0.01)	0.91	1.91 (±1.10)
WWW (11)	1.04 (±0.09)	1.00 (±0.06)	0.027 (±0.01)	1.00	2.80 (±0.90)
Control (11)	1.06 (±0.16)	1.20 (±0.16)	0.025 (±0.02)	0.89	3.20 (±1.90)

* numbers in parenthesis refer to the number of days after planting that the treatment was terminated

Average shoot water potential of the seedlings in DD was -1.5 MPa at planting (Table 4.3). The seedlings in DD were planted into dry soil and were wilting on the day after planting. Although the seedlings in both the dry soil treatments (DD and WD) were visibly wilting three days after planting, shoot water potential was much lower for the seedlings in DD (-2.80 MPa) than WD (-1.65 MPa) at the termination of these treatments (Table 4.3). Seedlings in the Control, WD, WWD and WWW, were well watered the day before planting resulting in an average shoot water potential of -0.93 MPa (Table 4.3). For seedlings in WWD and WWW, shoot water potential remained steady for the first three days after planting, followed by a drop to -1.4 MPa between days three and seven. For seedlings in WWD, shoot water potential remained at -1.4 MPa until trial termination. Following re-watering of seedlings in WWW, at nine days, shoot water potential increased to -0.87 MPa (Table 4.3). The shoot water potential in the Control was low (-0.46 MPa) at trial termination, (Table 4.3).

Table 4.3. Average shoot water potential (MPa) of seedlings used in a pot trial implemented to determine the effect of water availability at, and after, planting on *P. patula* water stress. Values in brackets are the standard deviation of the mean.

Time relative to planting (dap*)	DD	WD	WWD	WWW	Control
-1	-1.50 (±0.20)	-0.93 (±0.10)	-0.93 (±0.10)	-0.93 (±0.10)	-0.93 (±0.10)
1	-	-1.60 (±0.20)	-0.88 (±0.13)	-0.88 (±0.13)	-
3	-2.80 (±0.41)	-1.65 (±0.14)	-0.83 (±0.06)	-0.83 (±0.06)	-
7	-	-	-1.40 (±0.14)	-1.40 (±0.14)	-
11	-	-	-1.40 (±0.13)	0.87 (±0.24)	-0.46 (±0.19)

* dap refers to the number of days since the planting day which was considered as day 0.

Throughout the trial, measurements of shoot water potential related well to that of stomatal conductance. Conductance was highest for the Control, where soil water was not-limiting and shoot water potential was high (Figure 4.2 and Figure 4.3). Small changes in stomatal conductance in the Control reflected changes in the vapour pressure deficit (Figure 4.2). Stomatal conductance for the seedlings in DD and WD declined rapidly following planting, and stomata were closed when the treatments were terminated (Figure 4.2). There was no plant available soil water in these treatments. Soil water content in WWD and WWW declined from 40% to just below 30% three to seven days after planting (Figure 4.3). During this period, conductance declined from 67% to 24% of that of the Control (Figure 4.2) and shoot water potential declined from 0.83 MPa to -1.40 MPa respectively. On day nine, despite low soil water content, stomatal conductance of seedlings in WWD and WWW increased, possibly in response to the reduced vapour pressure deficit (Figure 4.2). That the seedlings responded to reduced vapour pressure deficit, despite low soil water, indicates that critical soil water thresholds had not been reached. Following watering of the seedlings in WWW, there was a corresponding increase in conductance and shoot water potential (Figure 4.2). This was in contrast to the seedlings in WWD, where conductance was approaching 0 mmol m^{-2} s^{-1} at trial termination and it is likely the stomata had closed (although unlikely that the seedlings were dead). At this stage shoot water potential of the seedlings in WWD was -1.4 MPa and soil water had declined to below 30%.

Measurements of chlorophyll fluorescence, and derived JIP-test parameters, did not reflect the imposed water stress treatments. These data was therefore not explored any further.

4.4 DISCUSSION

4.4.1 *P. patula* and water stress

Based on the results of this study it was difficult to determine when and if mortality occurred in any of the treatments. Plants can remain green with their water potential declining over a long period after being physiologically dead (Kaushal and Aussenac, 1989). As this trial was conducted over a relatively short period of 11 days it meant that the determination of critical water stress thresholds was difficult. Since the seedlings in DD and WD were not re-watered, it could not be determined if they had either reached the lower limits of water potential or if they were physiologically dead. Future studies on

P. patula will need to critically assess this. Kaushal and Aussenac (1989) defined the lower limit of water potential, before the onset of death, as the lowest potential obtained by the plant between transplanting and bud break, above which bud break occurred.

The DD and WD treatment provided some insight into the importance of root plug moisture at the time of planting. Soil water content in both of these treatments was very low (< 20%), yet visible symptoms of stress were delayed two days in WD. At termination of these treatments, average shoot water potential of seedlings in WD was also much higher than that in DD, -1.60 MPa versus -2.80 MPa, and it is possible that recovery may have occurred in WD on rewatering. During a commercial planting operation, especially when dry planting in summer, an extra two days could be critical to survival. The measurements of water stress of the seedlings in WWD also indicate the potential benefits of adding water to the pit when planting into dry soil (in terms of improved water availability and establishment of good root to soil contact).

High air temperature has been suggested as a possible cause of *P. patula* stress and subsequent mortality (Allan and Higgs, 2000). Survival of *P. patula* has been found to decrease when the mean daily maximum air temperatures exceed 22.5°C in the two weeks after planting, or 30°C for more than 10 days in the first month after planting (Morris, 1990; Allan and Higgs, 2000). Post-planting temperatures for the period covered by this study were extremely high, yet there was no mortality in the Control, WWD and WWW. This is probably a combination of the short duration of the study and the availability of soil water (it is likely that due to declining soil water content, mortality would have occurred in WWD soon after trial termination). Seedlings in WWW were able to recover quickly from a water stress of two to three days, despite simultaneous exposure to high temperatures. Seedlings in the Control, showed no indications of heat stress and maintained high levels of conductance (and transpiration) throughout the trial. This indicates that *P. patula* can tolerate moderately high air temperatures provided there is adequate access to soil water. High stomatal conductance and transpiration rates were found to be the primary mechanisms for avoiding heat damage in *P. ponderosa* seedlings (Kolb and Robberecht, 1996). Carlson *et al*. (2004) also found that high temperature stress over a short period (10 days) alone did not cause mortality in *P. patula* seedlings and suggested that water stress may drive mortality in this species. Water stress may also predispose the seedlings to fungal infections. Water stressed *P. resinosa* seedlings were more likely to be infected with *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton (syn. *Diplodia pinea* Desmaz.) than seedlings either not water stressed or treated with an appropriate fungicide (Stanosz *et al*., 2001). The role of water stress and the interaction with infection with disease, especially *Fusarium circinatum*, should be investigated for *P. patula* seedlings.

In the short term, application of water at planting (WWD and WWW), and even continuously moist soil (Control), did not affect the development of roots and shoots. However, the presence of water affected the temperature of soil in the root zone. Studies have indicated that optimum root growth occurs in soils with temperatures of 20 to 25˚C, with growth declining at higher temperatures (Lopushinsky and Max, 1990). Carlson *et al*. (2004) found that daily exposure of *P. patula* seedlings to soil temperatures above 24˚C, for at least one hour over a period of 10 days, significantly reduced the development of new roots. Field soil temperatures above 25˚C have been recorded in the root plug zone (Rolando and Little, 2004). The application of water at planting can reduce maximum root zone temperature and this may increase the rate of root regeneration in the days following planting.

4.4.2 Suitability of equipment

Chlorophyll fluorescence measurements were taken to determine if fluorescence measures could be used as indicators of water stress in pine regeneration research. Measurements of chlorophyll fluorescence have the potential to be quick, non-destructive, repeatable and accurate. In addition, preliminary research on *Eucalyptus grandis* seedlings had indicated the potential of this tool to detect water and light induced stress (Rolando and Little, 2003). Previous studies testing the potential of this equipment for stress detection on pines in South Africa have also yielded variable results, possibly a function of the physical difficulties associated with measurement of the small, primary needles (Rolando and Little, 2004). Fluorescence data collected in this study therefore confirmed previous perceptions as to the difficulties associated with this tool for water stress detection in young *P. patula* seedlings.

This study indicated that both the pressure chamber and the porometer provided a reliable measure of water stress. Since the needles of the seedlings were too small to be measured with the pressure chamber, measurements of the water potential of the excised shoot had to be made which meant that all sampling was destructive. Measurements of stomatal conductance were made with a chamber that enclosed part of the seedling and subsequent calculations required periodic determination of the needle area of the sample. This indicates that while either the porometer or pressure chamber may be used for field moisture stress measurements, most studies will require the planting of extra seedlings for destructive sampling.

4.5 CONCLUSION

Together with providing a practical assessment of the measurement of water stress in *P. patula* seedlings, this study has provided insight into the response of this species to water stress. It has also generated many questions. Further studies should aim to either improve our understanding of the physiological sensitivity of *P. patula* to water stress, or test the efficacy of applied treatments for minimizing post-planting water stress. Fundamental type studies should be aimed at understanding the effects of prolonged water stress on root regeneration, hydraulic conductance and the deployment of carbohydrates. Applied studies should be aimed at understanding the role of planting method (including the depth of planting, the application of water or hydrogels and handling), seedling quality (including seedling size, nursery conditioning and nutrition) and season of planting in reducing post-planting water stress and mortality.

CHAPTER 5

WATER STRESS IN CONTAINERISED *PINUS PATULA* SEEDLINGS FOLLOWING PLANTING: I. EFFECTS ON GROWTH AND PHYSIOLOGY

ABSTRACT

The effect of soil water availability following planting on growth and physiology of *Pinus patula* seedlings planted into pots was examined. The objectives of the study were to; 1) provide key physiological and morphological data for pine seedlings when exposed to varying levels of soil water stress after planting, 2) investigate the difference between two families in terms of root growth from the time of sowing and as affected by soil water availability after planting, 3) quantify changes in shoot water potential, stomatal conductance and transpiration in response to changes in soil water availability after planting, and 4) investigate the relationship between seedling morphological characteristics at planting and the water stress response (up to one month after planting). Measures of plant-water relations, root and shoot growth, soil water availability and air and soil temperature were made. There were no significant differences between the two families in growth and physiology in response to soil water availability following planting. Despite exposure to air temperatures above 35˚C, vapour pressure deficits above 4 kPa and low soil water availability, critical levels of water stress were not reached and no mortality occurred. The lowest shoot water potential, measured 23 days after planting, was just below -1.2 MPa. This indicated that the species is potentially more tolerant to drought stress than previously thought. Stomatal closure commenced at a shoot water potential of between 0.8 and 0.9 MPa. No relationship was found between measures of initial seedling morphology and subsequent root growth and physiology in response to soil water stress. However, measured rates of transpiration were found to be related to the mass of new roots produced during the month following planting.

5.1 INTRODUCTION

Pinus patula is the most widely planted softwood species in the summer rainfall region of South Africa (DWAF, 2005). Unacceptable mortality of this species following planting is a concern and has the potential to effect future deployment of this species for commercial timber. Applied research carried out to understand the impact of silvicultural and site management factors has indicated heat stress, pests and diseases and the management of harvest residues as having an influence on mortality (Morris, 1990; Bayley, and Kietska, 1997; Allan and Higgs, 2000; Allan *et al*., 2000; Crous 2005; Rolando and Little, 2005). While this research has improved silvicultural practices, there is still an inadequate understanding of the direct causes of mortality and the effects of non-optimal environmental conditions on seedling physiology and growth immediately after planting. This is coupled with a general lack of studies, both locally and internationally, investigating the morphological and physiological characteristics of *P. patula* seedlings (Rundel and Yoder, 1998; Oviedo and Emmingham, 2003). Excepting for three studies (Bayley and Kietska, 1997; Carlson *et al*., 2004; Rolando and Little, 2008a) no literature has been published on the ecophysiology of this species during regeneration in South Africa. In order to improve our understanding of factors driving early survival and growth for *P. patula* there is a need for more fundamental, physiological type studies. Understanding how this species responds to different environmental stresses, particularly water stress, may provide an indication of the type of stock required to meet the demands of the site, as well as increase our understanding of the effects of early silviculture on mortality.

In the native range of *P. patula*, in the mountainous regions of tropical Mexico, annual precipitation varies between 1000 mm to 2500 mm and mist is common, occurring between 42 and 176 days per year (Richardson and Rundel, 1998). Average daily temperatures range from 10 to 18˚C (Dvorak *et al*., 2000). Sites considered optimum for *P. patula* in South Africa are generally over 1000 m above sea level, receive more than 850 mm annual rainfall and have mean a annual air temperature of less than 18˚C (Dvorak *et al*., 2000; ICFR, 2005). As the species is planted on a wide range of sites, however, climatic conditions during the summer planting season (September to March), even on sites considered suited to *P. patula*, can be extreme. Maximum daily air temperatures can range from extremely hot (over 30˚C) to cool (below 15˚C) within a few days, and rainfall can be sporadic, with no rain occurring for several days, to weeks, followed by short, intense rainstorms (Schulze, 1997; Rolando and Little, 2007). Water planting (application of water into the planting pit at the time of planting) of *P. patula* seedlings has been used commercially, to reduce post-planting water stress and buffer against potentially extreme weather conditions immediately after planting (Morris, 1994; Allan *et al*., 2000; Oscroft *et al*., 2000). However, the application of water does not always increase survival (Rolando and Little, 2007; Rolando and Crous, 2008) highlighting the lack of understanding of the ecophysiology of this species.

Rolando and Little (2008a) investigated equipment suitable for the measurement of water stress in young pine seedlings (seven months) subjected to different levels of soil water availability over a period of 11 days. Although this trial was limited in terms of sample size, the results indicated that planting *P. patula* seedlings into wet or dry soil played a role in survival during the period immediately following planting. Building on this study, a more comprehensive pot trial was initiated to; 1) provide key physiological and morphological data for pine seedlings (where none has existed before) when exposed to varying levels of soil water stress after planting, 2) investigate the difference between two families in terms of root growth from time of sowing and as affected by soil water availability after planting, 3) quantify changes in shoot water potential, stomatal conductance and transpiration in response to changes in soil water availability after planting, and 4) investigate the relationship between seedling morphological characteristics at planting and the response to water stress (up to one month after planting). Two families were included in the study to allow for an evaluation of the impact of seed source on root growth and physiology in response to environmental conditions.

5.2 MATERIALS AND METHODS

5.2.1 Description of trial and treatments

The trial was carried out at the Institute for Commercial Forestry Research (ICFR) nursery, Pietermaritzburg. The pots used in the trial were chosen to best reflect the dimensions of standard planting pits used for planting pines in the KwaZulu-Natal Midlands, South Africa. This was based on data collected for a study on commercial pitting standards in KwaZulu-Natal where the average size of pits was 28 cm wide by 18 cm deep, with an average volume of approximately six litres (Rolando and Little, 2006). The pots (25 cm diameter x 15 cm deep) were filled with the equivalent of four litres of air dried soil (3.9 ±0.16 kg soil per pot). The soil used to fill the pots was a clay texture (66% clay, 10% silt, and 24% sand) collected from the Linwood plantation, located in the KwaZulu-Natal Midlands (29° 33' 57" S; 30° 06' 08" E). Permanent wilting point occurs at a soil matric potential of approximately -1.5 MPa and for this soil at low bulk density occurs when the gravimetric soil water content (kg kg^{-1}) drops below about 0.28 (or 28%) (Smith *et al*., 2001). Field capacity (generally accepted to be the soil moisture content at a matric potential of -10 kPa) will be attained at a gravimetric soil water content of about 0.38 (or 38%) (Smith *et al*., 2001). A polystyrene base was used to insulate the pots from ground temperatures as well as to prevent entry of ground-dwelling fauna into the pot-soil. The area where the trial was carried out was similar to an open field, with no nearby trees or large buildings. As all the watering in the trial was controlled, the pots were covered with shelters during rainfall events to prevent inadvertent wetting of the soil (and seedlings). *P. patula* seeds from two different families (AP006 and AP176) were sown into polystyrene trays filled with a pine bark and coir mix on the 30th May 2006. An excess of seed was sown to ensure availability of seedlings for all destructive measurements as well as the pot trials. The pot trial was initiated approximately six months later on 20th November 2006. A sample of 80 uniform seedlings (in terms of height and groundline diameter measurements) from each seed source was selected to be used in the pot trial. Average height and groundline diameter for seedlings from the two seed sources at trial initiation was 11.0 cm and 12.7 cm, and 1.6 mm and 1.9 mm, for AP176 and AP006 respectively.

The trial consisted of a factorial combination of 8 treatments (2 x 4), each replicated 20 times (each replicate consisted of a single seedling planted in a pot). The treatment factors were family (2 families) and water stress (4 levels of water stress were imposed on the seedlings). The water stress treatments were designed to simulate different conditions of soil water availability during the month following planting (Table 5.1; Figure 5.1). Air temperature (1.5 m above ground) and relative humidity were measured every 30 minutes for the duration of the trial with an Onset Hobo® logger (Onset Computer Corporation) housed in a Stevenson Screen. Vapour pressure deficit (VPD in KPa) at the daily maximum temperature was derived from measurements of air temperature and relative humidity (Unwin, 1980). The trial was terminated on the 15th December 2006, 24 days after initiation.

Table 5.1. Description of the four water stress treatments used in a pot trial to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families.

Water stress level	Treatment description
None (Control)	Planted into moist soil with soil kept moist (at field capacity) for the duration of the trial, (water with 1 litre every 2 days).
Mild	Planted into moist soil and watered with 1 litre every 4 days
Moderate	Planted into moist soil and watered with 1 litre every 8 days
Severe	Planted into moist soil and watered with 1 litre every 12 days

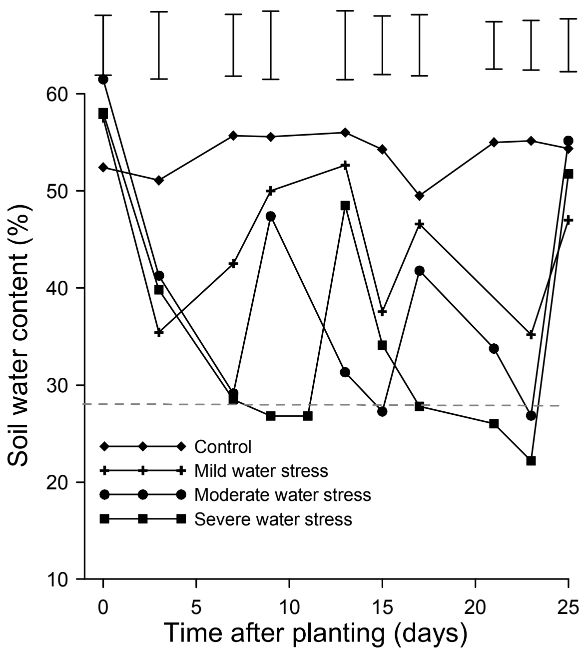


Figure 5.1. Changes in gravimetric soil water content (0.06 m below soil surface) for the duration of a pot trial implemented to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Line at 28% indicates estimated soil water content at wilting point (-1.5 MPa) for clay soils at low bulk densities (Smith *et el.*, 2001). Bars represent the standard errors of the mean.

5.2.2 Seedling measurements

The height (ht in cm), groundline diameter (gld in mm), root and shoot oven dry mass (g) of 25 randomly selected seedlings per family was assessed at 16, 20 and 24 weeks following sowing (in the nursery). The heights and groundline diameters of the seedlings selected for the trial were measured immediately after planting as well as at termination of the trial. At termination the oven dry mass of new roots emerging from the plug, as well as total shoot and needle mass of the seedlings was determined (new shoot mass was not determined due to the difficulties in identification there of).

Measurements of stomatal conductance (G_s in mmol m^{-2} s^{-1}) and transpiration (E in mmol m^{-2} s^{-1}) were made with a LI-1600 Steady State porometer fitted with a cylindrical chamber recommended for measurement of conifers, with standard operating procedures followed (LICOR, 1984). As the chamber was too small to enclose the entire seedling, only the top 2 to 3 cm of each seedling was inserted into the chamber. A stable displayed resistance was taken to indicate that a null-balance had been achieved. Measurements were made on all seedlings in the morning (8h00-9h00) and around mid-day (12h00-13h00) on the day of planting, and on regular days thereafter. A chromel-constantan thermocouple attached to the sensor head, and in contact with the needles during the conductance measurements, was used to measure needle temperature (˚C). A quantum sensor (LI-190S-1) attached to the porometer was used to measure photosynthetic photon flux density (PPFD in :mol m^{-2} s^{-1}), (LICOR, 1984). The sensor was oriented vertically above seedlings during the measurement. Needle area was estimated by scanning the measured needles and determining total area with an image analysis programme. The needles were removed from the shoot so that limited overlap occurred.

Measurements of shoot water potential (MPa) were made using the pressure chamber technique (Scholander, 1965). Since the primary needles were too small, measurements were made on the excised stem (with needles) and were therefore destructive. Predawn measurements (05h00) were made on three seedlings of each treatment and each family when treatments were most likely to reflect the imposed stress level. This was on days 7, 15 and 23 (after planting) for the control, mild and moderate stress treatments, and days 11, 15 and 23 for the severe water stress treatment. These seedlings were also used to determine the mass of new roots emerging from the plug during the course of the trial. An assumption of this study was that the unharvested seedlings had the same mean shoot water potential as the harvested seedlings, and an indirect correlation between shoot water potential measurements and other measurements is therefore suggested.

5.2.3 Measurements of soil temperature and soil moisture

Measurements of the soil temperature in the zone of the root plug (0.10 m, below the soil surface) were made from the time of planting until trial termination. These temperature measurements were made with copper-constantan (Type T) thermocouples connected to a Campbell Scientific CR10x datalogger that used a 10TCRT thermistor for the reference temperature (Campbell Scientific, 1997). An AM416 Multiplexer (Campbell Scientific,

1997) was used to increase the number of thermocouples that could be scanned by the CR10x. One thermocouple was placed at each measurement point in five pots for each treatment. Temperature measurements were logged every hour and were based on an average of measurements taken every two minutes.

Measurements of the volumetric water content (m^3 m^{-3}) of the soil, 0.06 m below the surface, were made with a Delta-T Theta Probe type ML2 (Delta-T Devices Ltd) (Little *et al*., 1996). Data were calibrated for the soil in the pots using gravimetric soil moisture content samples (Little *et al*., 1996). Using the probe, measurements of soil water content were taken in each pot for all treatments on the day of planting and on each date that measurements of stomatal conductance or shoot water potential were made.

5.2.4 Analyses

Comparisons between families for measures of seedling morphology made prior to treatment initiation were made using the Students *t*-test (Steel and Torrie, 1980). Subsequent comparisons between family and water stress treatment means for morphological (ht, gld, shoot and new root dry mass) and physiological (conductance, transpiration, leaf temperature and shoot water potential) variates were made using a factorial analysis of variance for each measurement date. Only if the *F*-test was significant were the means further investigated using the least significant difference statistic (LSD) (Steel and Torrie, 1980). Where no significant differences were found the analyses have not been presented. Correlation and regression were also used to further explore the data and understand the relationships between initial and final measurements of growth and morphology as well as the relationship of the measures of physiology with seedling morphology. Where necessary, variates were transformed using the appropriate method (Gomez and Gomez, 1976). All analyses were conducted using Genstat® for Windows™ Version 9 (Payne *et al*., 2006).

5.3 RESULTS

5.3.1 Air and soil temperature measurements

The trial was conducted during summer (November to December) when mid-day air temperatures, light intensity and vapour pressure deficits were high (Figure 5.2a and 5.2b). Maximum air temperature exceeded 35˚C on 7 days, and 30˚C on 16 days during the trial.

Maximum pot soil temperature in all treatments exceeded 35˚C on 16 days and was as high as 50˚C on two occasions in the severe water stress treatment (Figure 5.3). Pot soil temperature was higher in all treatments in comparison to the control, at all ambient temperatures, with the highest temperatures occurring where soil water content was lowest (severe water stress). Differences in maximum soil temperatures between the control and severe water stress treatments were more pronounced (up to 10˚C) during very hot weather (Figure 5.3). Minimum pot soil temperatures were similar for all treatments throughout the trial (Figure 5.3).

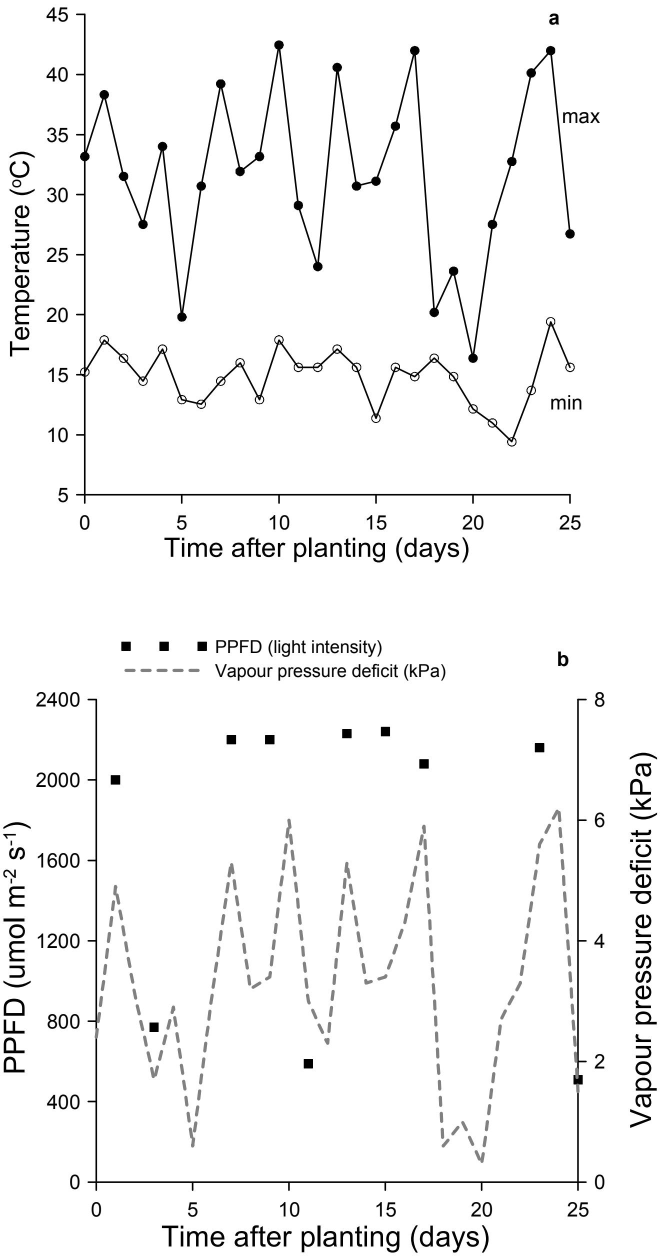


Figure 5.2. (a) Maximum and minimum air temperature and (b) light intensity (PPFD) and vapour pressure deficit (VPD) at maximum daily temperature for the duration of a pot trial implemented to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Light intensity was not measured on days 18, 20 and 21.

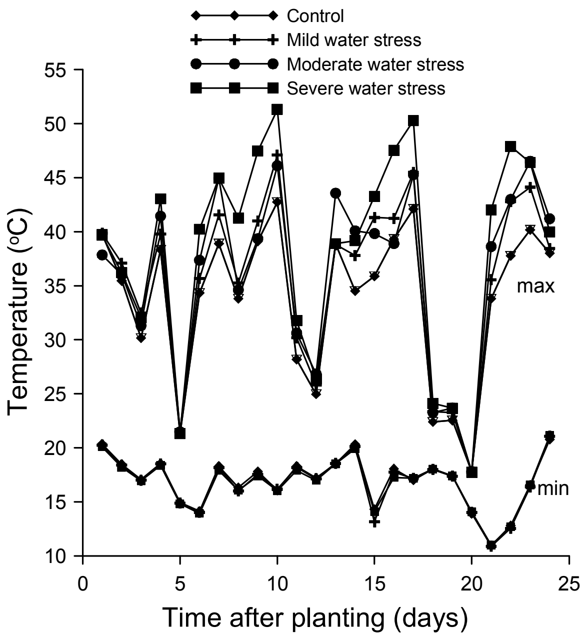


Figure 5.3. Maximum and minimum pot soil temperature (0.10 m, below the soil surface) for the duration of a pot trial implemented to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families.

5.3.2 Seedling growth

Nursery phase (16-24 weeks)

In terms of an increase in the absolute dry mass of roots and shoots, the two families (AP006 and AP176) showed similar development in the nursery (Figure 5.4a). Throughout this period, partitioning of plant biomass to roots was higher for AP176, and partitioning of biomass to shoots was higher for AP006, a trend that continued for the duration of the trial across treatments, as indicated by the consistently higher root:shoot ratio for AP176 (Table 5.2; Figure 5.4a and 5.4b).

Transplanting (trial initiation) to termination of trial

For both families, the highest mass of new roots was produced in the control and mild water stress treatments and the lowest mass in the severe water stress treatment (Table 5.2). The greatest increase in root mass occurred between the moderate and mild treatments (possibly representing a threshold in response to soil water availability) (Table 5.2). At trial termination the percentage increase in mass of new roots was 96, 87, 46 and 39% for the control, mild, moderate and severe water treatments (Table 5.2). Total shoot mass was similarly affected by the water stress treatments where there was a 108, 98, 76 and 44%

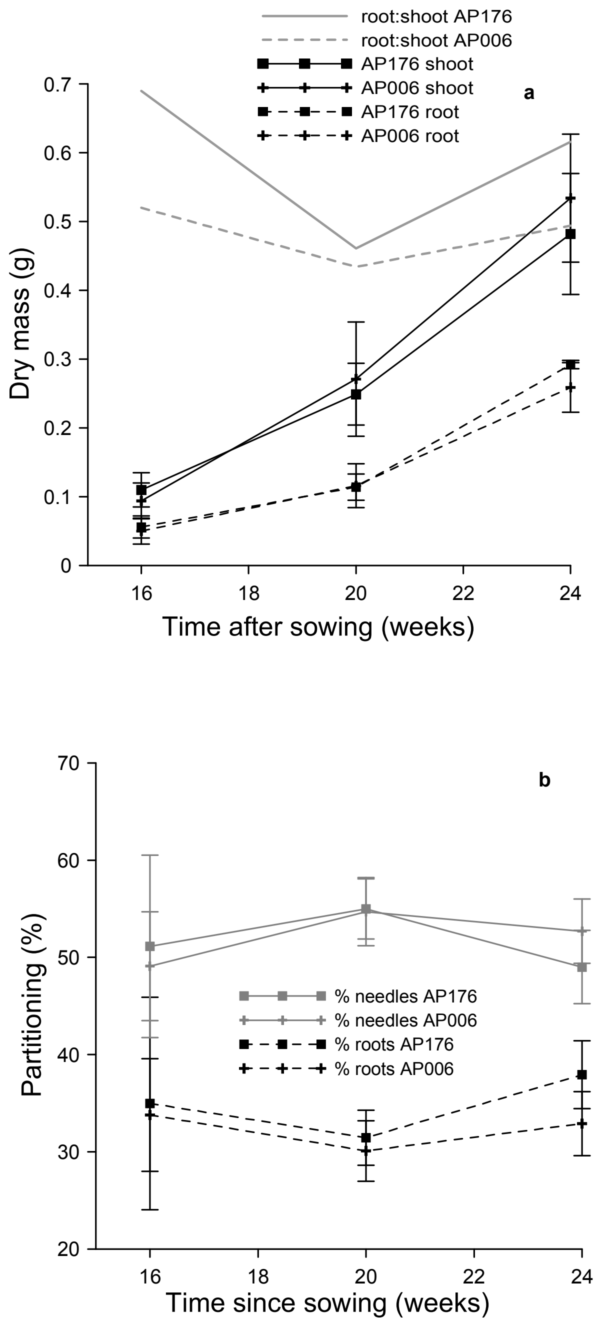


Figure 5.4. (a) Change in dry mass of shoots and roots and root:shoot ratio and (b) percentage biomass partitioning to needles and roots at 16, 20 and 24 weeks after sowing for two *P. patula* families used in a pot trial to determine the effect of soil water availability on growth and physiology during the month following planting. Bars represent the standard errors of the mean.

increase in total shoot mass from initiation to termination. Although absolute shoot and root mass increased more in the control and mild water stress treatments (Table 5.2), allocation of new biomass to roots and shoots was similar across treatments as indicated by the consistency of the root:shoot ratio from the time of planting for each family (Table 5.2).

Table 5.2. Total shoot and root mass (g) at planting and at trial termination (new root mass shown) in a pot trial to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Values in brackets are the standard deviation of the mean and numbers followed by different letters in each row of the table are significantly different (p<0.05).

Family	At planting	At trial termination			
		Control	Mild	Moderate	Severe
	Root mass (g)	**New root mass (g)**			
AP176	0.29 (0.04)	0.29 (0.05)a	0.24 (0.09)a	0.12 (0.05)b	0.11 (0.03)b
AP006	0.26 (0.04)	0.24 (0.04)a	0.24 (0.08)a	0.13 (0.06)b	0.11 (0.05)b
Total shoot mass (g)					
AP176	0.48 (0.09)	1.02 (0.08)a	0.89 (0.14)a	0.84 (0.08)b	0.70 (0.09)c
AP006	0.53 (0.09)	1.08 (0.17)a	1.12 (0.19)b	0.94 (0.14)b	0.75 (0.12)c
Root:shoot					
AP176	0.60	0.56	0.59	0.49	0.56
AP006	0.49	0.46	0.45	0.41	0.49

There was no significant effect of family on new root mass across water stress treatments and therefore these data were pooled to determine the effect of the different soil water treatments on new root growth for the duration of the trial. Divergence between the root growth curves occurred at approximately 15 days (Figure 5.5a) after planting, when both the moderate and severe treatments had received only one litre of water since planting. The effect of soil water availability on the mass of new roots was evident in the increase in the percentage variance accounted for by the regression of root mass on soil water content over time (Figure 5.5b). By 25 days there was a clear affect of water availability on the mass of new roots.

The mass of new roots produced by the seedlings in each treatment was not significantly correlated to measurements of initial size (height or groundline diameter measurements). However, final root mass was related to final measurements of height and groundline diameter where seedlings with a greater height and groundline diameter had a greater mass of new roots regardless of treatment.

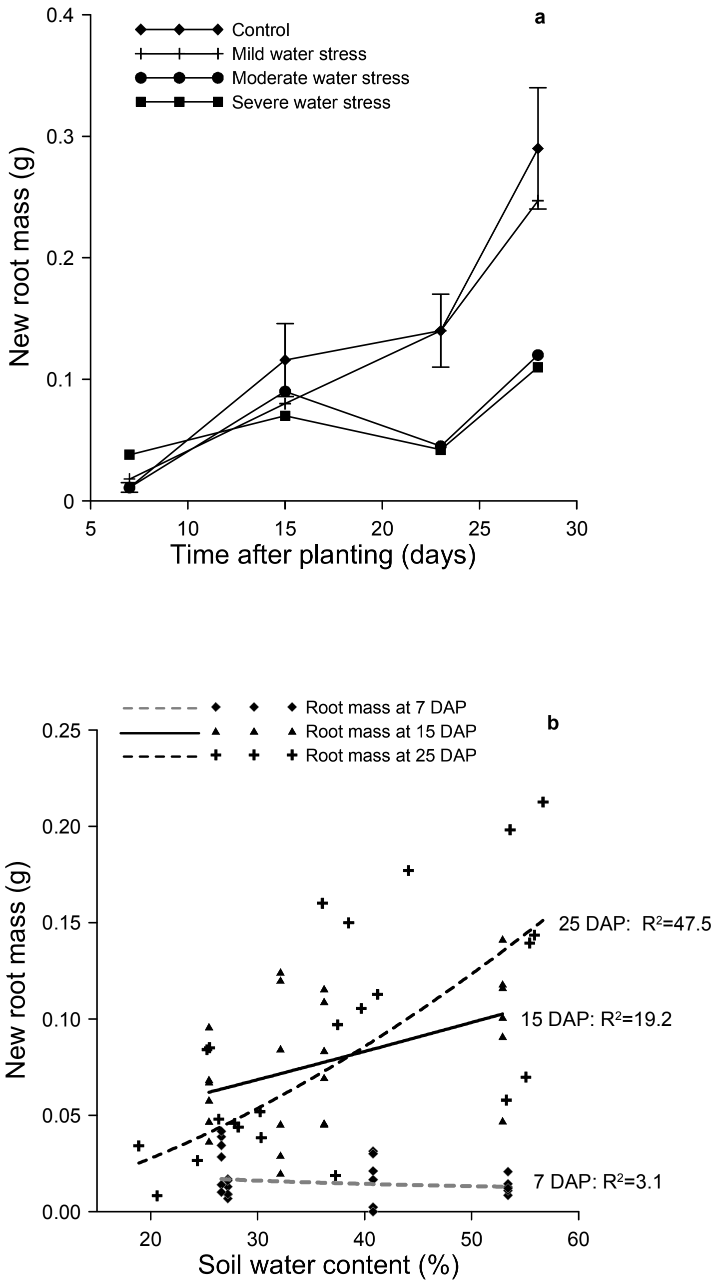


Figure 5.5. (a) Mass of new roots produced over time in response to four soil water treatments. Bars represent the standard errors of the mean. (b) Simple linear regression was used to show the change in percentage variance accounted for (R^2) by soil water content (affected by the various treatments) on mass of new roots over time. DAP refers to days after treatment initiation. Each point represents an individual seedling at a specific point in time (7, 15 or 25 DAP).

5.3.3 Seedling physiology

There were no significant differences between families for measurements of shoot water potential for the duration of the trial. Significant differences in shoot water potential between the water stress treatments were detected at each measurement date (Table 5.3; Figure 5.6). The lowest average shoot water potential in the severe water stress treatment was only just below -1.2 MPa, despite low soil water content between days 9 to 12 and 21 to 23 (Figure 5.1). This was surprising as these seedlings had received only one litre of water since trial initiation, three weeks earlier, and it was expected that a higher level of water stress would be imposed. Since there were no significant differences between families for measurements of shoot water potential, these data were combined for each treatment for further analysis.

The data were further explored to determine whether measurements of shoot water potential within and between treatments could be related to measures of seedling morphology (height and groundline diameter measurements and the mass of new roots produced at the time of sampling). This was carried out to investigate potential seedling quality attributes that could contribute to a greater tolerance of post planting water stress. No significant relationships were found.

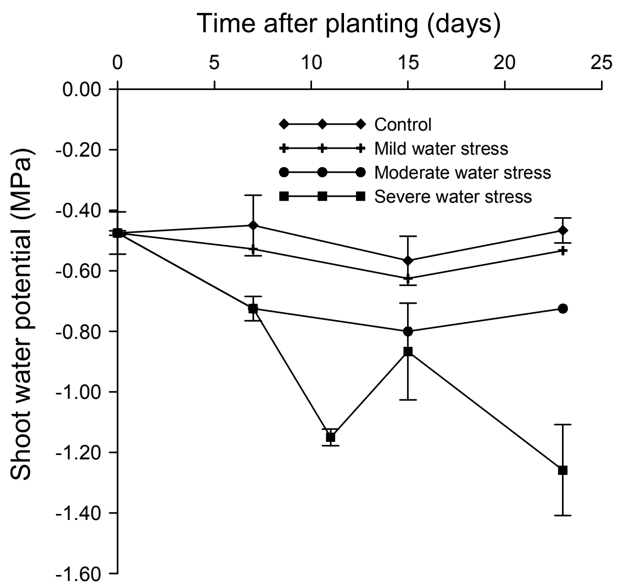


Figure 5.6. Changes in shoot water potential (MPa) with time in response to changes in gravimetric soil water content (%) for each treatment for the duration of a pot trial implemented to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Bars represent the standard errors of the mean.

Table 5.3. Summary of the ANOVA's for shoot water potential (MPa) and conductance (mmol m^{-2} s^{-1}) measurements made on days 7, 11, 13, 15, 17 and 23 in a pot trial to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families.

	Mean squares												
	Shoot water potential (MPa)				Conductance (mmol m^{-2} s^{-1})								
Source of variation	df	Day 7	Day 15	Day 23	df	Day 11 AM	Day 11 PM	Day 13 AM	Day 13 PM	Day 17 AM	Day 17 PM	Day 23 AM	Day 23 PM
Family	1	52267	75938	10417	1	84.3	71.4	2.4	7.7	237.7	45.4	380.3	1135.7*
Treat	3	588767*	120660*	772361*	3	17426.3*	36916.2*	25613.0*	12692.1*	30133.7*	8516.2*	22924.5*	14952.5*
Family.Treat	3	27656	23715	17917	3	440.7	59.5	13.4	20.0	871.6	575.2*	2342.1*	1160.9*
Residual	16	42663	11771	10521	40	121.6	195.8	162.8	178.5	5576.5	148.8	343.5	535.2
Total	23				47								
Grand mean		-0.7	-0.7	-0.7		100.7	81.6	77.2	42.5	43.8	47.4	44.2	26.5

* significant at $p<0.05$

For most measurement days there were no significant differences between the two families for the water stress treatments for measures of stomatal conductance in the morning (8h00-9h00) and at mid-day (12h00-13h00) (Table 5.3). Where significant differences between families did occur they accounted for a small percentage of the variation in comparison to the water stress treatment effect (Table 5.3). These data were therefore pooled to present the change in stomatal conductance across water stress treatments over time (Figure 5.7a-d). Morning conductance was generally higher than that at mid-day especially on hot days for the control and mild water stress treatment.

Conductance measurements for the mild water stress treatment were similar to the control in the morning for the duration of the trial, but were significantly lower for the afternoon measurement from 9 days (Table 5.4). Although lower than the control, soil water content for the mild water stress treatment at 0.06 m below the soil surface was above the wilting point (Figure 5.1), and shoot water potential was above -0.65 MPa, for the duration of the trial (Figure 5.6). For the moderate water stress treatment, morning conductance and afternoon conductance measurements were significantly different from the control from 9 days after planting (Table 5.4; Figure 5.7c). Shoot water potential was between -0.7 and -0.8 MPa from 5 days after treatment initiation (Figure 5.6) and estimated soil water content (0.06 m) around the wilting point (28%) on days 7, 15 and 23 after planting (Figure 5.1). Complete stomatal closure for this treatment occurred 23 days after planting, on a hot day with a high vapour pressure deficit when soil water content (0.06 m) was at its lowest (23%) and shoot water potential was -0.725 ±0.08 MPa (Figure 5.2a and 5.2b; Figure 5.6; Figure 5.7c). Morning and mid-day stomatal conductance for the severe water stress treatment was also significantly different from the control from 9 days after planting (Table 5.4; Figure 5.7d) and mid-day conductance measurements for the moderate and severe water stress treatments were not significantly different from days 15 to 23. Stomatal closure occurred on days 17 and 23, both hot days with high vapour pressure deficits (Figure 5.2a and 5.2b). Shoot water potential dropped to -1.2 MPa on days 11 and 23, the lowest recorded for the trial. Soil water content (0.06 m) dropped to the estimated wilting point from days 9 to 11, and from days 21 to 23 (Figure 5.1). Following the final watering of all treatments 24 days after planting, the three water stress treatments recovered with morning conductance significantly higher than that of the control (Figure 5.7a-d). Measured rates of transpiration (and conductance) were variable, but this could be partly explained by variation in individual seedling root mass. Seedlings with a greater mass of new roots tended to have higher transpiration rates, at any point in time, especially in the mild and moderate water stress treatments (Figure 5.8).

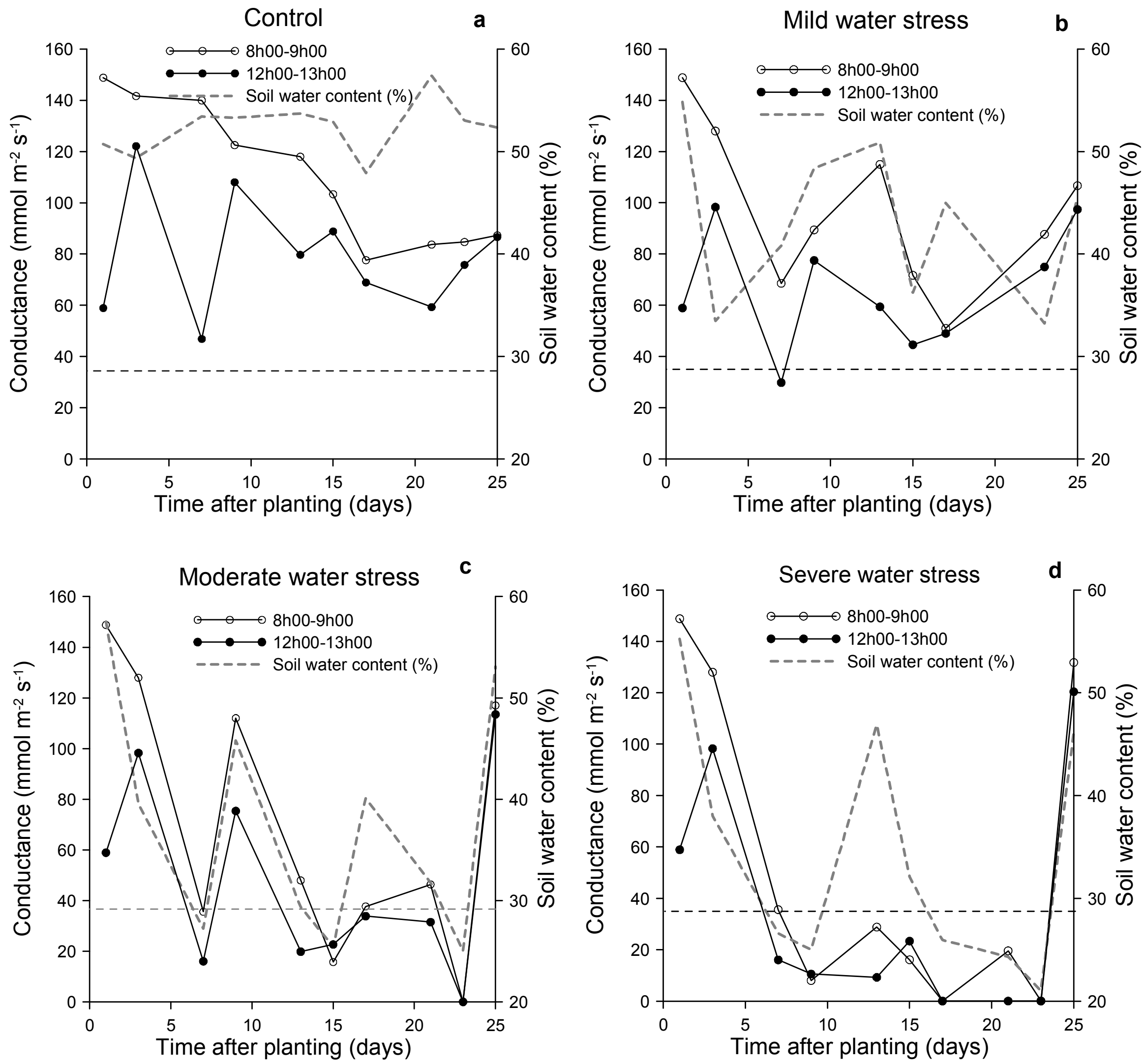


Figure 5.7. Morning (08h00-9h00) and mid-day (12h00-13h00) stomatal conductance (mmol m^{-2} s^{-1}) for the four water stress treatments in a pot trial implemented to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Line at 28% represents the estimated soil water content at wilting point for clay soils at low bulk densities.

Table 5.4. Treatment means for the conductance measurements made in the morning and at mid-day on days 9, 11, 13, 17 and 23 for the four water stress treatments applied in a pot trial to determine the effect of soil water availability during the month following planting on growth and physiology of *P. patula* seedlings from two families. Within each row numbers followed by different letters for each measurement date are significantly different ($p<0.05$).

Day	Control	Mild	Moderate	Severe
Morning				
9	114.8a	95.0b	96.7b	15.1b
11	115.1a	121.6a	122.4a	43.8b
13	118.0a	115.0a	48.8b	27.1c
17	73.8a	57.1b	37.9c	6.4d
23	81.2a	82.2a	13.1b	0.1b
Afternoon				
9	90.0a	76.9b	69.1b	12.8c
11	121.7a	109.9b	94.6c	0.1d
13	80.0a	58.4b	22.0c	9.5d
17	80.0a	58.4b	25.5c	25.8c
23	74.6a	31.3b	0.1c	0.1c

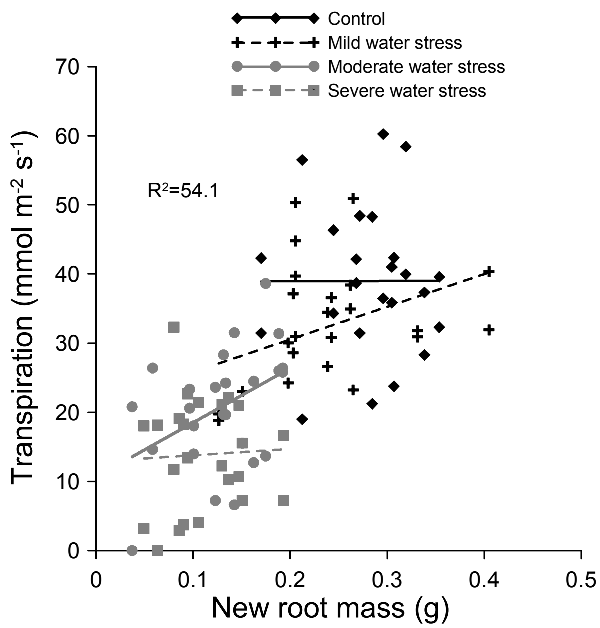


Figure 5.8. The relationship between rate of transpiration and new root mass for the seedlings in each of the treatments for both families. The data represent a simple linear regression with groups (the different treatments) and the regression was significant at $p<0.01$.

There was a significant effect of water stress on needle temperature measured both in the morning and at mid-day (data shown for mid-day measurements only; Table 5.5). On most measurement days morning and mid-day needle temperatures were higher in the moderate and severe water stress treatments in comparison to the control (Table 5.5). This difference was pronounced on hot days where differences greater than 5˚C were recorded.

Table 5.5. Treatment means for needle temperature of *P. patula* seedlings made at mid-day (12h00-13h00) in response to four water stress treatments. Within each row numbers followed by different letters for each measurement date are significantly different ($p<0.05$).

Day	Control	Mild	Moderate	Severe
9	30.1a	30.8b	32.0c	33.5d
11	24.5a	24.6a	25.2b	25.9c
13	34.9a	36.1b	38.0c	38.2c
17	35.8a	37.5b	39.3c	40.5d
23	34.6a	36.6b	39.0c	40.2d

5.4 DISCUSSION

The lack of consistently significant differences between families in measures of morphology and physiology meant that most of these data were pooled for further analyses. While this may only be a reflection of the chosen families, it may be that the genetic variability of *P. patula* in South Africa, for the measurements carried out in this study, is narrow or that the genetic differences are made apparent only with maturity and in response to site and environmental conditions (and would therefore not be apparent in a controlled study). The only principal difference between the two families was found in the partitioning of root to shoot biomass during the nursery phase, reflected in a higher root:shoot ratio for the seedlings from the family AP176 throughout the trial. Biomass partitioning to above and below ground components in conifers has been shown to vary among families of loblolly pine, Douglas fir and among geographical sources of several other tree species (Merritt, 1968).

One of the objectives of the trial was to link measures of initial seedling size (ht and gld) to new root growth and measures of physiology. Since new root growth represents active accessing of additional resources, particularly water, it follows that seedlings with a high capacity for root growth would be less stressed under conditions of low soil water availability. However, no statistically robust relationships between measures of initial size (ht and gld) and subsequent root growth were found for the seedlings used in this trial. This may be a function of the uniformity of seedlings initially selected where the range in size may not have provided sufficient variability to detect significant differences with the precision required for the tests applied. However, a relationship between measured rates of transpiration and new root mass was detected. Kaushal and Aussenac (1989) reported on large individual plant variation for measurements of photosynthesis and transpiration, which they suggested may be attributed to root regeneration. This aspect would possibly be better tested on seedlings of various sizes exposed to soil water stress.

The most outstanding result from this study was that the level of imposed stress in the severe water stress treatment did not approach a critical threshold, as indicated by the shoot water potential measurements, as well as the lack of any mortality. The lowest measured shoot water potential, measured 23 days after planting into pots, was just below -1.2 MPa. This, despite very high soil and air temperatures in the severe treatment as well as several days where the measured soil water content reached estimated wilting point. Since only the upper 0.06 m of soil were measured by the Theta Probe it is possible that the soil moisture in the zone of the root plug (lower in the pot) was higher than that measured. This may explain the apparently high shoot water potentials when the measured soil water content was low. Despite this, these results have indicated that *P. patula* is possibly more tolerant to non-optimum environmental conditions than previously expected. This treatment was based on results from a previous pot trial where shoot water potential decreased to -1.4 MPa in response to low soil moisture, followed by near stomatal closure 8 to 10 days after transplanting into initially wet soil (Rolando and Little, 2008a). It was therefore estimated that 12 days with no watering would constitute a more severe stress and the rest of the treatments were structured relative to this.

Despite the trial failing in regard to estimation of critical water stress thresholds, at trial termination there was a significant difference in the mass of new roots produced by the control and mild treatment relative to that of the moderate and severe water stress treatment (possibly representing a threshold), as well as divergence between measures of stomatal conductance. This indicated that levels of soil water availability had imposed a degree of stress on the seedlings. Stomatal conductance has been experimentally shown to be related to net CO_2 assimilation rate, environmental vapour pressure deficit, soil water stress and intercellular CO_2 concentration (Fitter and Hay, 2002; Gao *et al.*, 2002). Some plants do, others do not, show midday depression of transpiration as a water conservation strategy. Morning stomatal conductance in the moderate and severe treatments diverged from that of the control 9 days after planting, when shoot water potential was estimated at -0.8 to -0.9 MPa. Stomata therefore responded to the lowering soil water content in these treatments. The rapid change in conductance in response to the lowering soil water content may partly explain the maintenance of shoot water potential between -0.7 and -1.2 MPa for these two treatments for the duration of the trial. Complete stomatal closure occurred only in the severe water stress treatment on two occasions when air temperature was up to 40˚C and vapour pressure deficit was over 4 kPa. Many investigations have shown that stomata remain fully open until a critical or threshold leaf water potential is reached (Whitehead, 1980); from this value, the aperture begins to narrow with further water loss and closure complete within about 0.5 MPa of the threshold. Hsiao *et al*., (1976) proposed generalised threshold values of -0.5 to -1.0 MPa for mesophytes and -1.0 and -2.0 MPa (and lower) for xerophytes. Gao *et al*. (2002) showed that for different functional vegetation types, pines were the most, and broad-leaved trees the least, resistant to increases in soil water stress, with shrubs in between. Gao *et al*. (2002) also found that of all the vegetation types assessed, stomatal conductance in pines had the strongest dependence on vapour pressure deficit. Stomatal conductance decreased more with increased vapour pressure deficit due to the low soil-to leaf hydraulic conductance, possibly because of the presence of tracheids only in their vascular systems. Transpiration rates and stomatal conductances for the Mexican pine species *P. greggii*, *P. patula* and *P. montezumae,* subjected to drought stress during the first few weeks of the growing season, were reduced when the predawn water potential was between -1.0 and -1.2 MPa (Vargas Hernandez and Munoz-Orozco, 1991). It is possible that had the severe water stress treatment been extended for a further 2 days, critical levels of water stress could have been reached.

High air temperature has been suggested as a possible cause of *P. patula* stress and subsequent mortality (Morris, 1990; Allan and Higgs, 2000). Survival of *P. patula* has been found to decrease when the mean daily maximum air temperatures exceed 22.5°C in the two weeks after planting, or 30°C for more than 10 days in the first month after planting (Morris, 1990; Allan and Higgs, 2000). Air and soil temperatures for the period covered by this study were extremely high, yet there was no mortality. Seedlings in the control and mild water stress treatments, showed no indications of heat stress and maintained high conductances (and transpiration rates) throughout the trial. This indicated that *P. patula* has the potential to tolerate moderately high air temperatures provided there is adequate access to soil moisture. High stomatal conductance and transpiration rates were found to be the primary mechanisms for avoiding heat damage in *P. ponderosa* seedlings (Kolb and Robberecht, 1996). As observed in this trial, Kolb and Robberecht (1996) found needles of seedlings exposed to high air temperatures to be cooler when high levels of conductance were maintained. Transpiration has been shown to dissipate up to one quarter of the heat absorbed by leaves (Larcher, 1983). Carlson *et al*. (2004) also found that high temperature stress over a short period (10 days) alone did not cause mortality in *P. patula* seedlings and suggested that water stress may drive mortality in this species. Water stress may also predispose the seedlings to fungal infections. Water stressed *P. resinosa* seedlings were more likely to be infected with *Sphaeropsis sapinea* (Fr.:Fr.) Dyko and Sutton (syn. *Diplodia pinea* Desmaz.) than seedlings either not water stressed or treated with an appropriate fungicide (Stanosz *et al*., 2001).

The impact of ambient air temperature and soil water availability on soil temperature in the field would not be as extreme as that recorded in the pot trial (up to 50˚C in the zone of the root plug). In a field trial designed to test the effect of soil water availability at planting on subsequent seedling survival, Rolando and Little (2007) found no significant effect of soil water availability on measured soil temperature in the field. However, soil temperatures of up to 25˚C were recorded in the zone of the root plug. The importance of soil temperature in the zone of the root plug relates specifically to the effect of temperature on the production of new roots. The production of primary lateral roots and new root tips by *Pinus* species is strongly influenced by root zone temperature, water availability and their interaction (Nambiar *et al*., 1979; Brissette and Chambers, 1992; Sword, 1996; Sword *et al*., 2005). The negative effect of water stress on new root growth for *P. palustris* has been found to increase at higher root-zone temperatures (>20˚C), (Sword, 1996). Carlson (2004) found that daily exposure of *P. patula* seedlings to soil temperatures above 24˚C, for at least one hour over a period of 10 days, significantly reduced the development of new roots.

5.5 CONCLUSION

Although this study did not determine critical water stress thresholds for *P. patula*, it has provided benchmark data against which further studies can be compared. This is a major step forward for regeneration and plant quality research for *P. patula* in South Africa. The results have also indicated that there were few differences in the two *P. patula* families tested in terms of their growth and development in the nursery, as well as their physiological response to different levels of soil water availability during the month following planting (into pots). In order to understand the critical limits of water stress tolerance and its interaction with seedling growth and development after planting further trials will need to be conducted.

CHAPTER 6

WATER STRESS IN CONTAINERISED *PINUS PATULA* SEEDLINGS FOLLOWING PLANTING: II. DETERMINATION OF CRITICAL STRESS LEVELS AND THEIR RELATION TO MEASURES OF SEEDLING MORPHOLOGY

ABSTRACT

To investigate the effects of water stress on *Pinus patula* seedling growth and physiology during regeneration, a trial was implemented to determine the shoot water potential at which mortality occurred and the relationship between measures of water stress (shoot water potential, stomatal conductance and transpiration) and seedling morphology (in terms of root and shoot biomass and measures of size). Measures of plant-water relations, root and shoot growth, soil water availability and air and soil temperature were made. The results indicated that *P. patula* seedlings were not sensitive to high air and soil temperatures (above 35˚C) and low soil water availability (below -1.5 MPa). The seedlings were able to tolerate low soil water availability for up to 21 days and recovered from severe water stress where shoot water potential was below -1.5 MPa. The critical water potential threshold for changes in stomatal conductance was in the region of -0.8 MPa to -0.9 MPa and stomatal closure occurred at a shoot water potential of between -1.8 to -2.1 MPa Mortality occurred when shoot water potential declined to below -3.0 MPa. There was variability between seedlings in their potential for survival and growth. Inherently bigger seedlings had a greater capacity for new root growth following planting. Mass of new roots was significantly and positively related to higher rates of transpiration under conditions of low soil moisture.

6.1 INTRODUCTION

In the forestry industry, quality seedlings are those that will meet a desired level of growth and survival following planting (South and Mexal, 1984; Mohammed, 1997). Seedling quality is directly related to genetic composition, size, vigour and environmental conditions during regeneration (Puttonen, 1989; Dunsworth, 1997). Understanding the effects of environmental stress (e.g. water, heat, nutrient availability, light) on seedling growth and physiology is therefore key to improving seedling quality in a nursery and for producing a target seedling that better matches the site for regeneration. In this regard, the role water stress in early growth and physiology of *Pinus patula* seedlings shortly after planting, and the interaction of this with seedling quality, needs to be investigated in conjunction with current planting practices. Water planting (application of water into the planting pit at the time of planting) of *P. patula* seedlings has been used in commercial planting operations to reduce post-planting water stress and buffer against potentially extreme weather conditions immediately after planting. Since the use of water in the planting operation is costly, it is essential that the effects of planting with water on early survival, growth and physiology are quantified.

Limited applied research to date has generally shown that there is not always a significant increase in survival of *P. patula* seedlings in response to planting with water (Rolando and Little, 2007; Rolando and Crous, 2008). In addition, in a pot trial designed to investigate root growth and physiology of *P. patula* seedlings as affected by seed source and water availability immediately after planting Rolando *et al*., (2008; Chapter 5) found that *P. patula* seedlings were more tolerant to non-optimum environmental conditions (heat and water stress) than previously thought. Despite soil and air temperatures above 35˚C, as well as several days where the measured soil water content at 0.06 m below the soil surface reached estimated wilting point, the lowest shoot water potential measured in their study was just below -1.2 MPa (Rolando *et al*., 2008; Chapter 5). This is contrary to results from research on *Eucalyptus* planting stock, where planting with water has been shown to have a significant impact on post-planting survival and seedlings are sensitive to imposed water stress (Viero and Button, 2007; Rolando and Little, 2008b). Rolando and Little (2008a) also found that there was high variability in the growth and physiological response of the individual *P. patula* seedlings to water stress. This result was important in terms of related commercial planting operations in South Africa where culling and (or) grading is not practiced and seedlings with inherent poor survival and growth capacities may be deployed to the field. Since new root growth represents active accessing of additional resources, particularly water, it follows that seedlings with a high capacity for root growth would be less stressed for water under conditions of low soil water availability.

To increase the understanding of the effects of water stress on *P. patula* seedlings during regeneration, the following still needed to be determined; 1) the shoot water potential at which mortality occurred, and 2) the relationship between measures of water stress (shoot water potential, stomatal conductance and transpiration) and seedling morphology (in terms of root and shoot biomass and measures of size). Since no major differences in the physiology and growth of two *P. patula* families were found in a previous trial conducted Rolando *et al*. (2008; Chapter 5), only one family was included in this study.

6.2 MATERIALS AND METHODS

6.2.1 Description of trial and treatments

The trial was carried out at the Institute for Commercial Forestry Research (ICFR) nursery, Pietermaritzburg. An excess of *P. patula* seed was sown into polystyrene trays filled with a pine bark and coir mix on the 22nd September 2006. On 16th February 2007, a random sample of 90 seedlings were planted into pots. The pots and soil used in this trial, as well as the positioning of the trial and the covering of pots to prevent rain wetting the soil, have been described by Rolando *et al*., (2008; Chapter 5). The seedlings were left to establish in moist soil for three weeks before the initiation of soil water stress treatments on the 12th March 2007. Average height and groundline diameter of the seedlings at the time of transplanting was 9.0 ±1.3 cm and 1.5 ±0.1 mm, respectively. The period between planting and trial initiation was to allow root growth into the soil beyond the immediate zone of the root plug, such that any changes in seedling physiology would better reflect soil water content in the pot than in previous trials. Chronologically all figures and analyses refer to the time of treatment initiation as time “0”.

The trial consisted of two watering treatments, each applied to 30 seedlings; a control (no water stress) and a severe water stress (no water further water) (Figure 6.1). The control was watered to field capacity at regular intervals. An extra 30 seedlings were planted in separate pots for the severe water stress treatment for the determination of critical levels of water stress. To determine this, sets of three seedlings were re-watered every two to three days, 21 days after treatment initiation (this starting point was determined by the first day that mid-day conductance of the severe water stress treatment was zero). This was to establish the point at which recovery from the imposed water stress did not occur. Conductance and shoot water potential measurements were used to determine this point. The trial was terminated after 35 days when mortality occurred in the severe water stress treatment. Air temperature (1.5 m above ground) and relative humidity were measured every 30 minutes for the duration of the trial with an Onset Hobo® logger (Onset Computer Corporation) housed in a Stevenson Screen. Vapour pressure deficit (VPD, kPa) at the daily maximum temperature was derived from measurements of air temperature and relative humidity (Unwin, 1980).

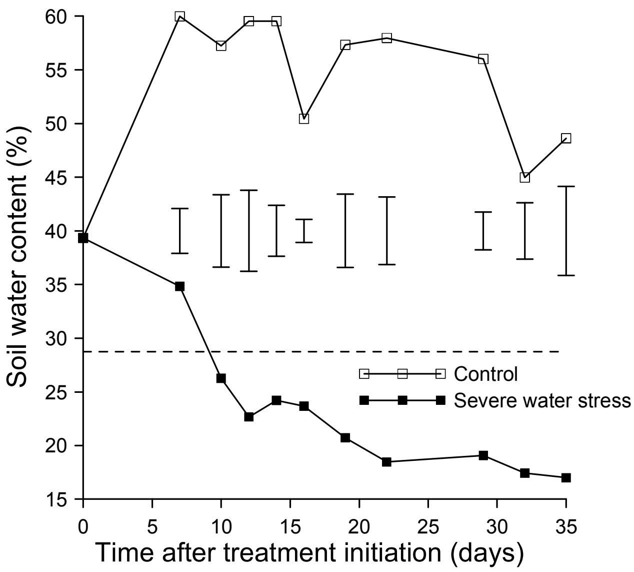


Figure 6.1. Changes in gravimetric soil water content in the zone of the root plug for the duration of the trial to examine the effect of soil water availability on *P. patula* survival, growth and physiology following planting. Line at 28% indicates estimated soil moisture at wilting point (-1.5 MPa) for clay soils at low bulk densities (Smith *et al.*, 2001). Bars represent the standard errors of the mean.

6.2.2 Seedling measurements

The height and groundline diameter of the seedlings in the pots was measured immediately after planting (ht_0 and gld_0), at the initiation of the water stress treatments three weeks later (ht_1 and gld_1) and at trial termination (ht_2 and gld_2). The oven dry mass of new roots emerging from the root plug, the length of the longest root as well as stem and needle biomass (above ground biomass) were determined during the course of the trial when seedlings were destructively harvested. Shoots and roots were dried at 60˚C for 72 hours prior to dry weight determination of total shoot weight and mass of new roots. As the destructive measurements were made periodically, any statistical analyses of seedling morphology required that the effect of time on growth be accounted for.

Measurements of shoot water potential (MPa) were made around mid-day (12h00-13h00) every two to three days on three seedlings of each treatment using the pressure chamber technique (Scholander *et al*., 1965) where measurements were made on the excised stem with attached needles. Since the entire above ground portion of the seedling was used to measure shoot water potential, it was not possible to observe mortality on the same plants used to measure water potential. An assumption that is implicit in the study therefore is that the unharvested seedlings had the same mean shoot water potential as the harvested seedlings, and an indirect correlation between shoot water potential measurements and other measurements is therefore suggested.

Measurements of stomatal conductance (mmol m^{-2} s^{-1}) were made every two to three days with a LI-1600 Steady State porometer fitted with a cylindrical chamber recommended for measurement of conifers (LICOR, 1984). As data collected from a previous pot trial (Rolando *et al*., 2008; Chapter 5) had indicated no additional understanding from measurements made both in the morning (8h00-9h00) and at mid-day (12h00-13h00), measurements were only made on seedlings (n=10) between 12h00-13h00. A chromel-constantan thermocouple attached to the sensor head, and in contact with the needles during measurements, was used to measure needle temperature. A quantum sensor (LI-190S-1) attached to the porometer was used to measure photosynthetic photon flux density (PPFD) in :mol m^{-2} s^{-1} at the time of mid-day measurements. Needle area was estimated by scanning the measured needles and determining area with an image analysis programme. The needles were removed from the shoot so that limited overlap occurred.

6.2.3 Measurements of pot soil temperature and soil moisture

Measurements of the soil temperature in the zone of the root plug (0.10 m, below the soil surface) were made from the time of planting until trial termination as described by Rolando *et al*., (2008; Chapter 5). One thermocouple was placed at each measurement point in five pots for each treatment.

Measurements of the gravimetric soil water content (g g^{-1} expressed as a percentage) of the soil in the zone of the root plug were made at each destructive sampling event to coincide with measurements of conductance and shoot water potential. This was carried out to ensure a more accurate estimation of soil water content in the root zone than in the previous pot trial (Rolando *et al*., 2008; Chapter 5).

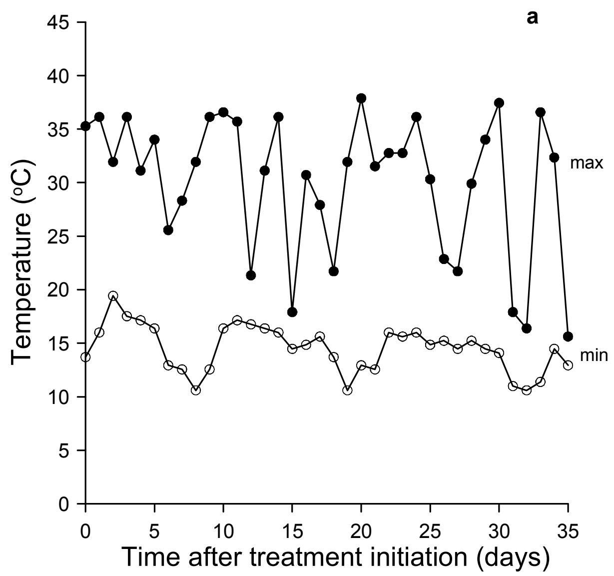
6.2.4 Analyses

Linear regression (Simple linear regression, Simple linear regression with groups and Polynomial regression with groups) was used to assess; 1) the effect of treatment on growth (in terms of root mass, shoot mass as well as explore relationships between measures of initial and final seedling size) and, 2) relationships between measures of seedling size (ht and gld) and seedling physiology (shoot water potential, stomatal conductance and transpiration) (McConway *et al*., 1999). Comparisons of treatment means for measures of shoot water potential and stomatal conductance were also made with the Student *t*-tests (Gomes and Gomez, 1976). Since most of the morphological and physiological measurements were made periodically, over time, it was necessary to account for the change in mass over time in some of the analyses. When necessary variates were transformed using the appropriate method (Gomez and Gomez, 1976). All analyses were conducted using Genstat® for Windows™ Version 9 (Payne *et al*., 2006).

6.3 RESULTS

6.3.1 Air and soil temperature measurements

The trial was conducted towards the end of the summer season (March to April) when maximum air temperatures, sunlight intensity and vapour pressure deficits were high (Figure 6.2a and 6.2b). Maximum air temperature exceeded 35˚C on nine days, and 30˚C on 24 out of the 35 days of the trial (Figure 6.2a). Soil water content in the control was above 45% for the duration of the trial while that in the severe water stress treatment fell below 28% (estimated wilting point) around nine to ten days after trial initiation (Figure 6.1). Maximum pot soil temperatures in the zone of the root plug were equally high, especially in the severe water stress treatment where the temperature exceeded 50˚C on two occasions (Figure 6.3). Differences in maximum soil temperature between the control and the severe water stress treatments were more pronounced on hot days and were as much as 10˚C on occasion (Figure 6.3).



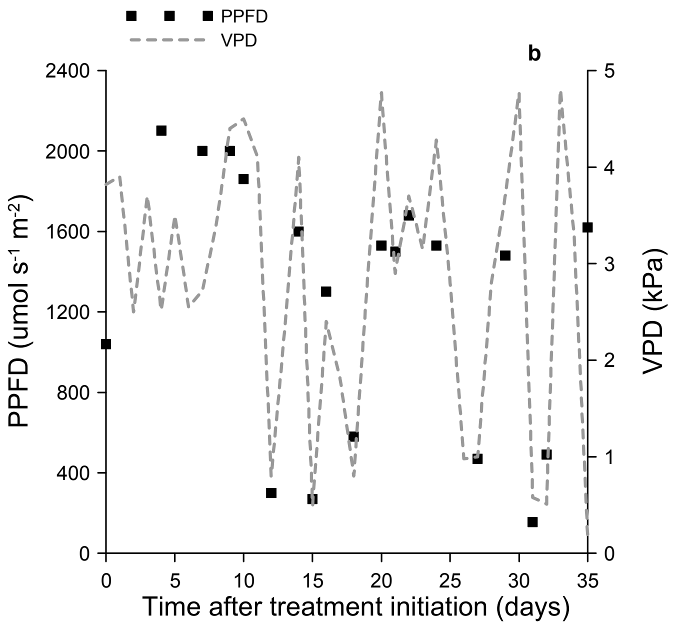


Figure 6.2. (a) Maximum and minimum air temperature, and (b) light intensity (PPFD) and vapour pressure deficit (VPD), for the duration of a pot trial to examine the effect of soil water availability on *P. patula* survival, growth and physiology following planting.

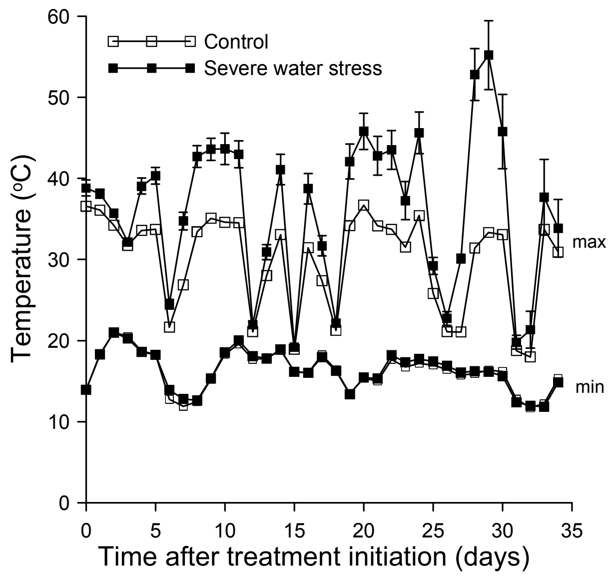


Figure 6.3. Maximum and minimum pot soil temperatures measured in the root plug zone (0.10 m below soil surface) for the duration of a pot trial to examine the effect of soil water availability on *P. patula* survival, growth and physiology following planting. Bars represent the standard errors of the mean.

6.3.2 Seedling growth

There was little increase in shoot mass from the time of treatment initiation to trial termination and no significant effect of the water stress treatment on shoot mass (Figure 6.4a). The above ground biomass increased from an average of 0.51 ±0.09 to 0.67 ±0.20 g (31%) for both treatments from the time of treatment initiation to termination of the trial. This corresponded with an average height and groundline diameter of 10.8 ±2.0 cm and 2.0 ±0.3 mm at termination.

At the time of treatment initiation, 21 days after planting the seedlings into the pots, the average length of the new roots was 4.6±0.02 cm and new root mass 0.045±0.02 g. At trial termination, 35 days after treatment initiation and 56 days after transplanting, the roots had grown a further 17.5 ±4.9 cm for the control and 13.5±6.6 cm for the severe water stress treatment. There was a significant and positive correlation between the length of the longest root and root mass (r=0.728; df=60, $r_{0.05}$=0.25). There was a significant effect of water stress on the mass of new roots produced during the course of the trial as indicated by linear polynomial regression (with groups) (Table 6.1a; Figure 6.4b). From the time of treatment initiation there was a 90% increase in total root mass in the control and a 45% increase in the severe water stress treatment. Production of new roots in the severe water stress treatment ceased between 18 to 20 days after treatment initiation. This corresponded to approximately 10 days growth where the gravimetric soil water content was below wilting point (Figure 6.1) and the point at which stomata closed.

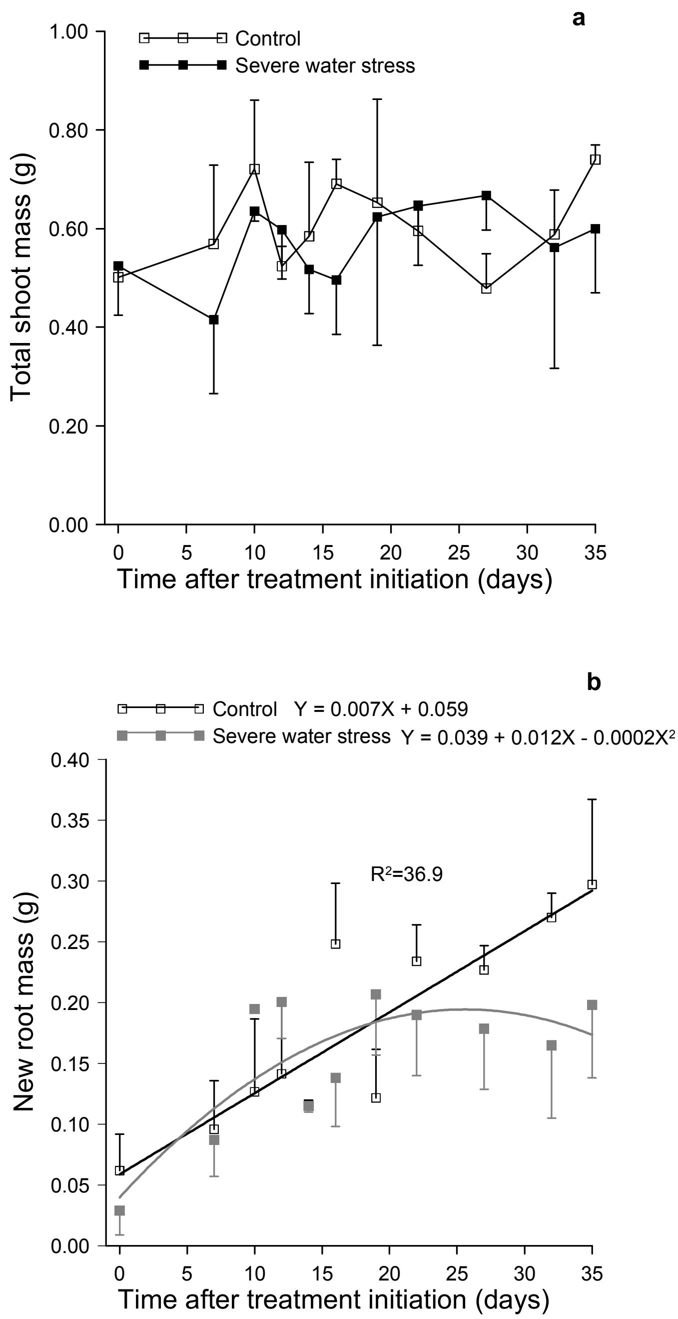


Figure 6.4. (a) Change in total shoot mass, and (b) mass of new roots during a pot trial to examine the effect of soil water availability on *P. patula* survival, growth and physiology following planting. The regression lines have been included to indicate the significant effect ($p<0.05$) of soil water stress on new root mass production over time. Bars represent the standard errors and for clarity have been shown in one direction only.

In terms of changes in biomass partitioning, there was an increase in partitioning of resources to root growth during the trial with a corresponding decrease in needle growth (Figure 6.5a). Root:shoot ratio was initially higher for the severe water stress treatment but this changed at approximately 15 days after treatment initiation (Figure 6.5b), possibly corresponding to the decrease in soil water content to below wilting point. Regardless of treatment, the mass of new roots produced by each seedling was also related to the measurements of initial height (ht_0) and groundline diameter (gld_0) made at the time of planting (Table 6.1b; Figures 6.6a and 6.6b). This effect, especially that of ht_0, became more pronounced with time (i.e. mass of roots produced by seedlings destructively harvested towards the end of the trial highlighted the strength of the relationship to initial measurements) (Figure 6.6a).

6.3.3 Seedling physiology

Chronological changes

Shoot water potential, stomatal conductance and transpiration responded to the changes in soil water availability from the time of treatment initiation (Figure 6.7a-c). T-tests indicated significant differences ($p<0.05$) in stomatal conductance between the control and severe water stress treatment commenced seven days after treatment initiation when shoot water potential was -0.867 MPa in both treatments (Figure 6.7a and 6.7b) and soil water content was 34% in the severe water stress treatment (Figure 6.1). From this point onwards, high stomatal conductance occurred in the severe water stress treatment only on days 12, 18 and 27, all cool days with low vapour pressure deficits (Figure 6.2a and 6.2b). Complete stomatal closure on days with high temperatures and light intensities first occurred in the severe water stress treatment 21 days after treatment initiation, when shoot water potential had declined to -2.0 MPa, and soil water content was 18.5 ±2.5%. This coincided with the point at which the production of new roots ceased (Figure 6.4b). However, even at this low soil water content and shoot water potential, stomatal conductance responded positively to low light intensity and low vapour pressure deficit 27 days after treatment initiation on cool days (Figure 6.2b; Figure 6.7b). At 32 days after treatment initiation shoot water potential in the severe water stress treatment had declined to an average of -3.5 MPa. At this point, the seedlings were showing signs of stress with tips wilting and the stomata were closed regardless of ambient temperature and light intensity (Figure 6.7a and 6.7b). On rewatering the seedlings in the severe stress treatment 32 days after treatment initiation one seedling did not recover from this low shoot water potential, indicating that the level of water stress reached was close to the limits of stress tolerance.

Table 6.1a. Summary of the linear polynomial (Pol) regression of new root mass during the course of the trial (Day) for the control and severe water stress treatment (Treat).

Regression		
Source of variation	**df**	**Mean square**
Regression	5	0.0274**
Residual	54	0.0036
Total	59	0.0056
Accumulated analysis of variance		
+Pol (Day;2)	2	0.0534**
+Treat	1	0.0027
+Pol (Day;2).Treat	2	0.0139*
Residual	54	0.0036
Total	59	

* significant at $p<0.05$ and ** significant at $p<0.01$

Table 6.1b. Summary of the linear regression with groups for the effect of Ht_0 and Gld_0 on new root mass measured weekly during the course of the trial (Week).

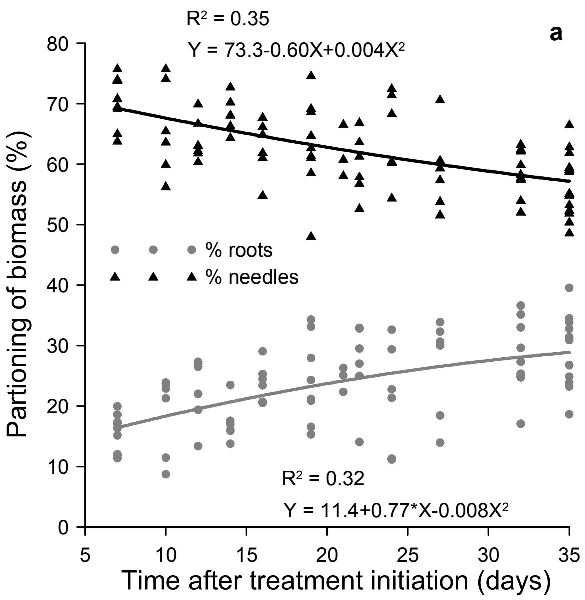
	Regression			
	Ht_0		Gld_0	
Source of variation	**df**	**Mean square**	**df**	**Mean square**
Regression	3	0.1144**	3	0.0782**
Residual	56	0.0042	56	0.0064
Total	59	0.0099	59	0.0100
Accumulated analysis of variance				
Ht_0/gld_0	1	0.0535**	1	0.0309**
Week	2	0.1449**	2	0.1019**
Residual	56	0.0042	56	0.0064
Total	59		59	

* significant at $p<0.05$ and ** significant at $p<0.01$

Table 6.1c. Summary of the simple linear regression analyses with groups for the effect of new root mass (explanatory variable) on rate of transpiration (dependant variable) for the control (n=9 for each measurement date) and severe water stress treatment (n=10 for each measurement date). Analyses were made on days (groups) with high light intensity (PPFD over 1500 mol m^{-2} s^{-1}). Days included in the regression were 4, 7, 10, 14, 20 and 24 for the control. The last measurement date was omitted in the regression for the severe water stress treatment as transpiration had ceased.

	Regression			
	Control		Severe water stress	
Source of variation	**df**	**Mean square**	**df**	**Mean square**
Regression	6	2209.9*	5	612.7**
Residual	47	55.9	44	12.4
Total	53	299.8	49	73.6
Accumulated analysis of variance				
+New root mass	1	4825.0**	1	973.5**
+Day	5	1686.8**	4	522.5**
Residual	47	55.9	44	12.4
Total	53		49	

* significant at $p<0.05$ and ** significant at $p<0.01$



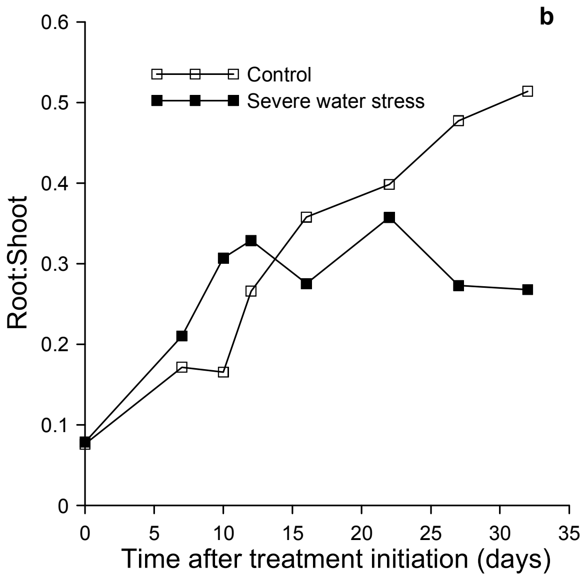
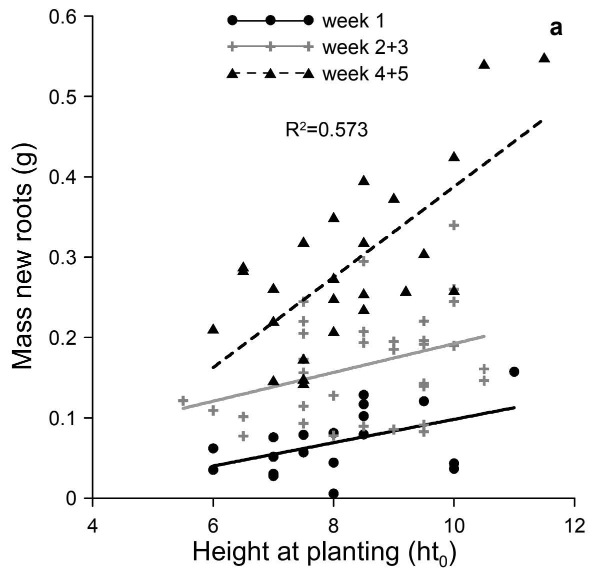


Figure 6.5. (a) Partitioning of biomass (%) to roots and needles and (b) root:shoot ratio for the control and severe water stress treatment during the pot trial implemented to examine the effect of soil water availability on *P. patula* survival, growth and physiology following planting.



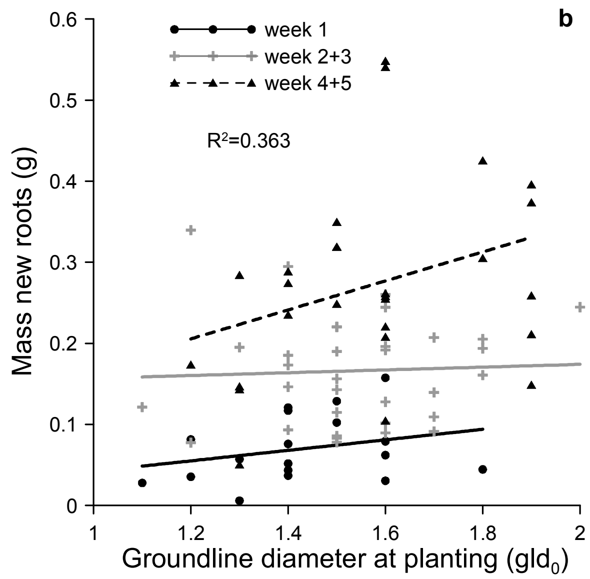


Figure 6.6. (a) The relationship of mass of new roots (when destructively harvested) to the measurement of initial height (ht_0), and (b) groundline diameter (gld_0) made on individual seedlings at the time of planting the seedlings into the pots. Since seedlings were destructively harvested over a period of five weeks, the weeks refer to the time after treatment initiation that the mass was determined. Linear regression of new root mass expressed as a function of the initial size measurement (ht_0; gld_0) with groups (Weeks), indicated a significant ($p<0.05$) effect of initial size and week of sampling on new root mass per seedling (Table 6.1b).

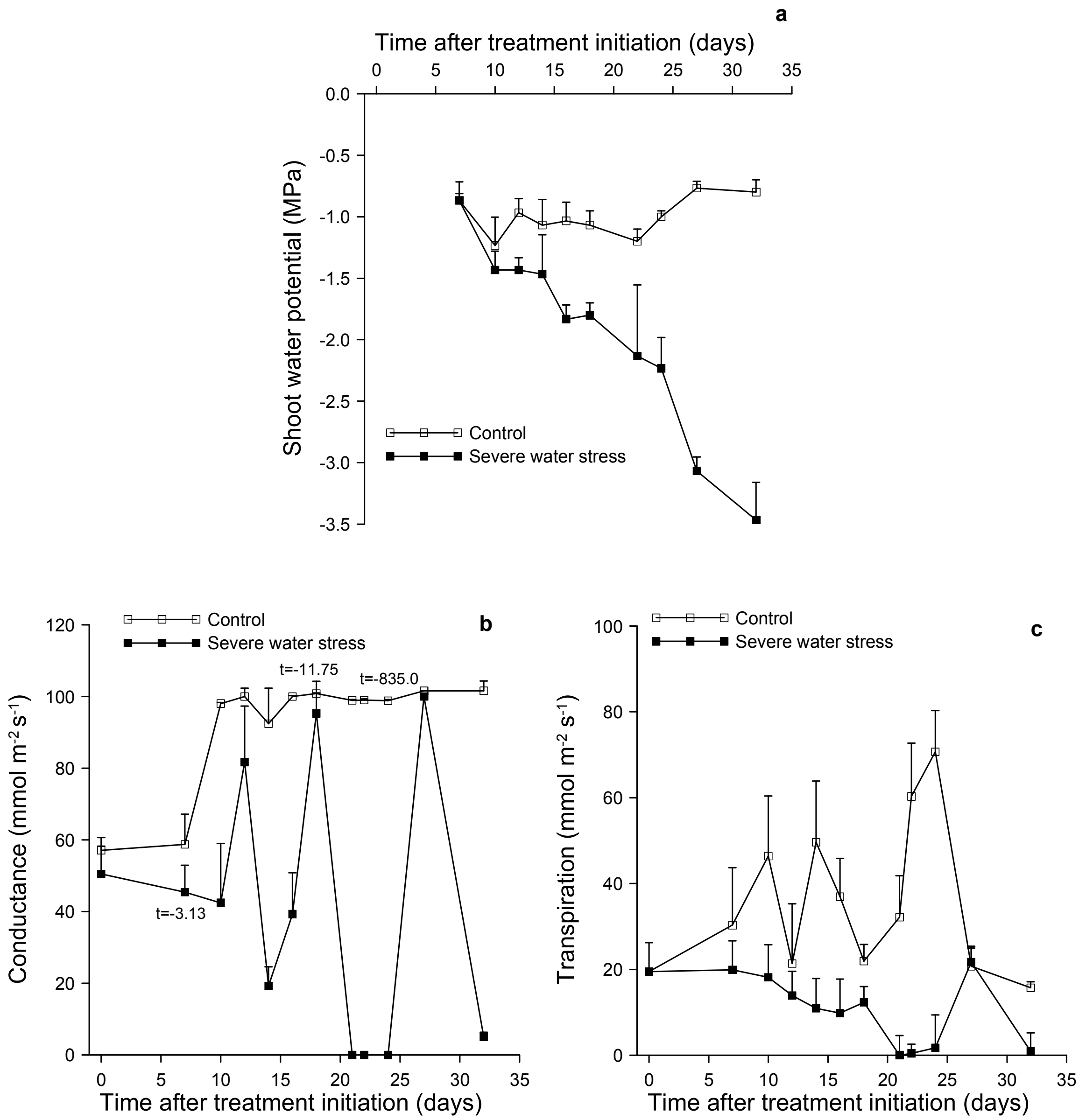


Figure 6.7. (a) Change in shoot water potential, (b) stomatal conductance and (c) transpiration for the control and severe water stress treatment during a pot trial implemented to examine the effect of soil water stress on *P. patula* survival, growth and physiology following planting. Bars represent standard errors and for clarity have been shown in one direction. Student *t*-tests (p<0.05) indicated significant differences between conductance measurements commenced seven days after treatment initiation. The calculated *t*-value has been included in (b) for days 7, 20 and 24 (for 18 df; $t_{0.05} = 2.101$).

Physiological changes in response to soil water content

Shoot water potential responded to changes in soil water content only when soil water content declined to around estimated wilting point (Figure 6.8a). This occurred between seven and 10 days after trial initiation (Figure 6.1) and coincided with a measured shoot water potential of -1.4 ±0.15 MPa at 10 days (Figure 6.8b). Further changes in shoot water potential occurred as the number of days soil water content remained below estimated wilting point accumulated (Figure 6.8b). The limits of water stress were reached by those seedlings exposed to soil water below 30% for more than 22 days, indicated by the non-recovery of one seedling when rewatered. All other seedlings recovered from exposure to low soil water contents between 0 and 22 days, including those where shoot water potential dropped below -2.5 MPa.

When soil water content was above 30% stomatal conductance was generally consistent, and high, despite changes in vapour pressure deficit, light intensity, leaf temperature and shoot water potential (Figure 6.9a-d). Only at high light intensities (2000 :mol m^{-2} s^{-1}) did conductance decrease slightly even when soil water was not limiting (Figure 6.9b). In contrast, when soil water availability was below 30%, there was a more definite stomatal response to these environmental variables (Figure 6.10a-d). Conductance generally decreased in response to high vapour pressure deficits, high light intensities, high leaf temperatures and shoot water potentials between -1.5 and -2.0 MPa.

Physiology and morphology

Measured rates of transpiration (and conductance) were variable, but this could be partly explained by variation in individual seedling root mass (Table 6.1c; Figure 6.11a and 6.11b). For the control, the relationship between mass of new roots (g) and transpiration (mmol m^{-2} s^{-1}) was evident only on very hot days, with a PPFD over 1500 :mol m^{-2} s^{-1} (days 4, 7, 10, 14, 20, 24), when rates of transpiration were high (Figure 6.11a). Differences in new root mass accounted for 81% of the variation in the data set (Table 6.1c). Seedlings with a higher mass of new roots were able to maintain higher rates of transpiration on these days. On cooler, overcast days, transpiration was unaffected by mass of new roots in the control treatment. In the severe water stress treatment this relationship was consistent at every measurement date, regardless of air temperature or PPFD (Figure 11b, data shown for PPFD over 1500 mmol m^{-2} s^{-1} only; Table 6.1c). That is, seedlings with a higher mass of new roots generally had higher rates of transpiration, regardless of air temperature, PPFD or level of water stress (only on day 24 when soil water content was low did mass of new roots not affect transpiration as the stomata had closed).

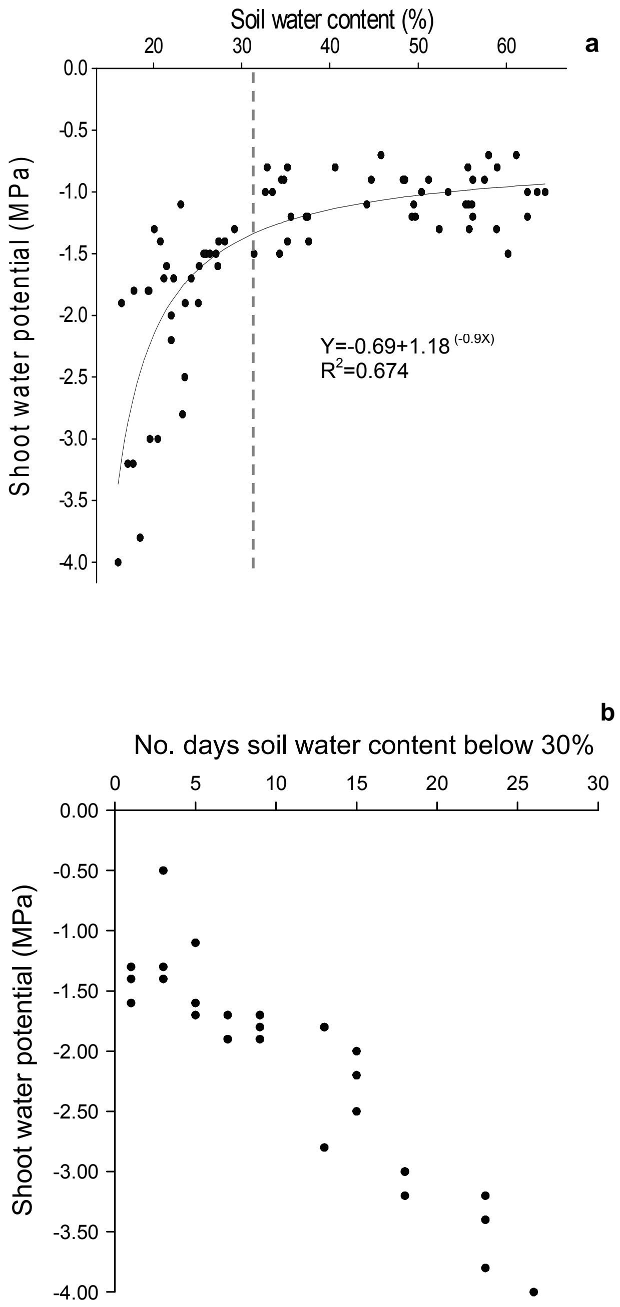


Figure 6.8. (a) Changes in shoot water potential in response to changes soil water content. Line at 28% represents the estimated soil water content at wilting point (-1.5 MPa) for clay soils at low bulk densities. (b) Changes in shoot water potential in response to the number of days where soil water content was below 30%. Only data where soil water content was below 30% for at least one day have been included.

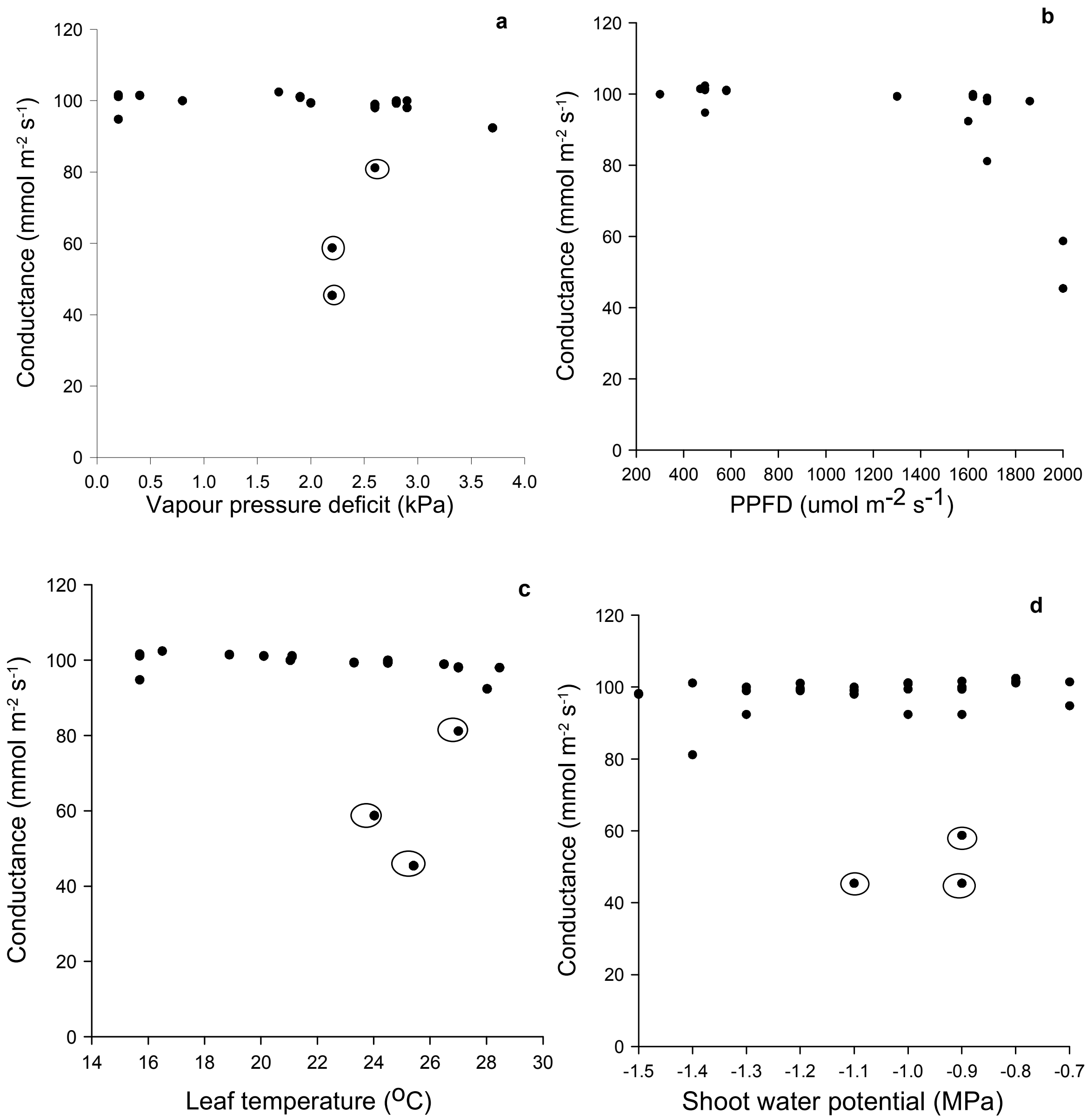


Figure 6.9. The effect of (a) vapour pressure deficit, (b) light intensity, (c) leaf temperature and (d) shoot water potential (MPa) on stomatal conductance of *P. patula* seedlings under conditions of high soil water availability (above 30%). Circled data points in figures a, c and d represent the conductance values recorded at high PPFD.

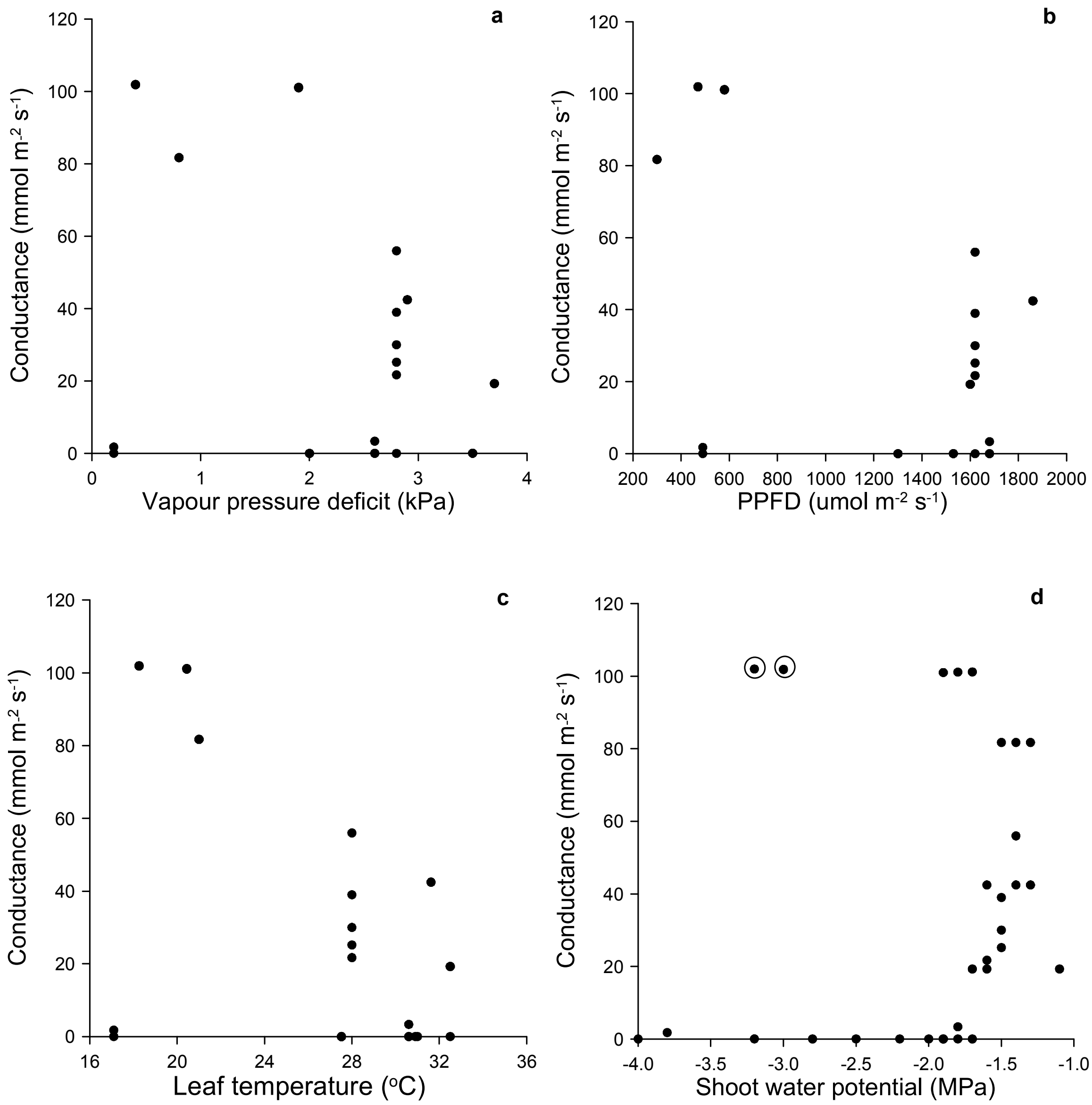


Figure 6.10. The effect of (a) vapour pressure deficit, (b) light intensity, (c) leaf temperature, and (d) shoot water potential on stomatal conductance of *P. patula* seedlings under conditions of low soil water availability (below 30%). Circled data points in figure (d) represent the conductance values recorded at very low PPFD despite low soil moisture.

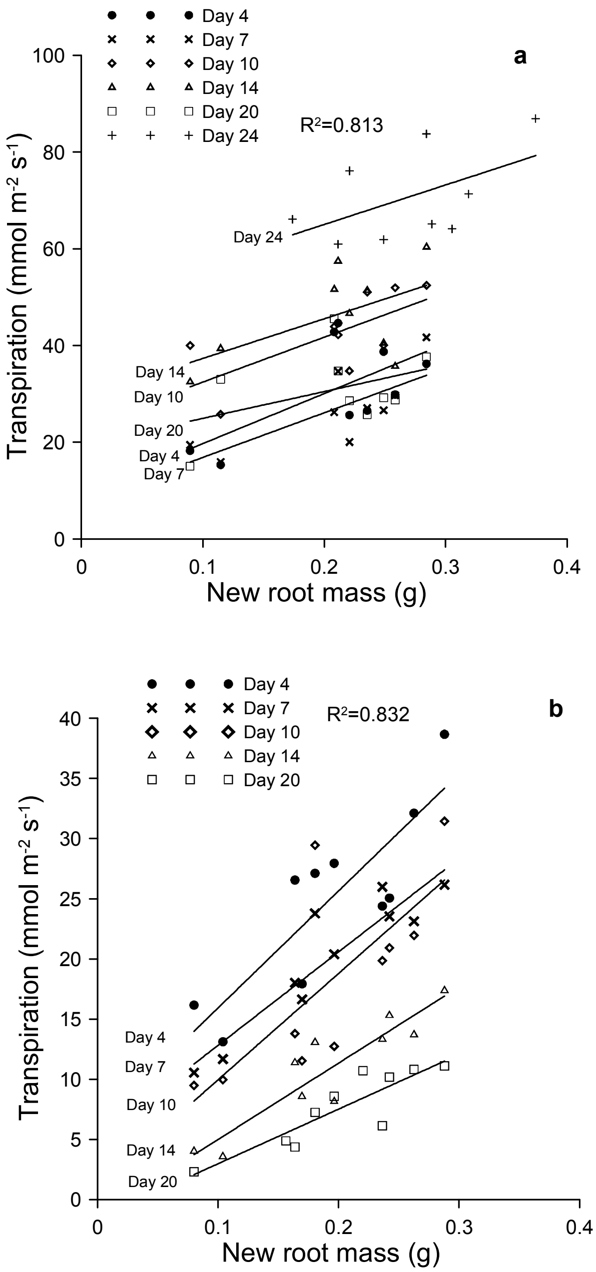


Figure 6.11. The relationship between the rate of transpiration and new root mass for seedlings in the (a) control treatment and (b) the severe water stress treatment. Only days where PPFD was above 1500 mmol m^{-2} s^{-1} were included. Transpiration had ceased on day 24 for the severe water stress treatment. Linear regression of transpiration and new root mass with groups (Day) indicated a significant effect (p<0.05) of new root mass on the measured rate of transpiration (Table 6.1c).

6.4 DISCUSSION

Root and shoot growth were slower in this trial than that measured in a similar trial with *P. patula* seedlings (Rolando *et al*., 2008; Chapter 5). There was also no apparent effect of soil water stress on shoot growth in this trial. Overall, little biomass was partitioned to shoot growth as was indicated by the decrease in partitioning of biomass to needles and the increase in the root:shoot ratio. Typically, root:shoot ratio increases when juvenile plants are under stress from water deficits as either above ground growth is affected more severely than below ground growth or an increase in partitioning to roots increases the efficiency of exploitation of available soil water (McMillin and Wagner, 1995). Mild water stress may also reduce leaf growth before photosynthesis resulting in a surplus of carbohydrates available for root growth (Boyer, 1970). Joly *et al*. (1989) interpret this as an adaptation that restricts transpirational surface area and increases water absorption from the soil. This trend was observed during the first 10 days where the root:shoot ratio was higher in the severe water stress treatment. Seasonal patterns in the root and shoot growth of trees are known to exist, where alternating allocation patterns, dependent on plant genotype and environmental factors, occur (McMillan and Wagner, 1995). Few studies have considered the effect of timing of water stress, relative to the growth dynamics of the plant, on biomass partitioning to roots and shoots. As early as 1968, Merritt (1968) emphasized that researchers should not ignore the effects that root growth periodicity might have on the response of tree seedlings to water treatments.

The average length of new roots at trial termination, 56 days after planting, was 17.5 cm in the control and 13.5 cm in the severe water stress treatment. From the perspective of field planting this would indicate that within two months of planting the roots would have extended to beyond the zone of the planting pit (a manually disturbed volume of soil 30 cm wide by 20 cm deep), if planted during late summer and into wet soil. The importance of root length in this regard pertains specifically to soil water availability in the pit (disturbed soil) versus that outside the pit (undisturbed soil), as once the roots have extended beyond the pit, then pit soil moisture may no longer be critical to survival. Since bulk density of the soil in the pit is lower than that outside of the pit (Bassett, 2008), soil water availability is likely to decline more rapidly in the pit. The sooner access to soil water reserves in undisturbed soil can be obtained, the more likely the seedling would be able to survive periods of water stress. The advantage of a higher soil water content in the pit would be to facilitate faster root growth, as indicated by the length of roots in the control treatment. In this regard, smaller (narrower but not necessarily shallower) planting pits would be ideal. Research to investigate the interaction of pit size with water availability has been initiated for commercially grown species, including *P. patula* (Viero *et al.*, 2008).

Exploration of the relationship between measurements of initial size (ht and gld) and subsequent mass of new roots indicated that a higher mass of new roots at trial termination was related to initially bigger seedlings. Relationships between initial seedling size and subsequent growth have been found in other studies carried out with *P. patula* in South Africa (Rolando and Little, 2005). Studies on *Pinus taeda* suggest that the survival of bareroot seedlings planted in the field is correlated with root length, root number and root:shoot biomass ratio, and that this is also variable within a population of seedlings (Kormanik *et al*. 1998). Kormanik *et al*. (1998) showed significantly lower growth in diameter at breast height, root collar diameter, total root biomass, top biomass and volume for plantation trees with lower first order lateral roots (FOLR) at the time of planting. This research highlighted the importance of heritability in the development of seedling quality. A system of grading seedlings at the time of planting is not used in South Africa. Grading may be difficult in a containerised system, where direct examination of the root system at planting is not possible. This, together with aspects pertaining to shoot size and root volume in a container, would need to be carefully optimised for a containerised nursery system such that size and age were not confounded. The negative affects of confined roots on subsequent seedling growth have been documented (South and Mitchell, 2006).

Variability in measurements of transpiration were also related to root mass, where seedlings with a greater mass of new roots showed higher rates of transpiration under conditions of high evaporative demand (as observed for the control treatment). This result indicates that there is potential for a system to identify seedlings that have a greater capacity for root growth and a subsequent capacity to tolerate water stress. Brissette and Chambers (1992) showed that both needle water potential and stomatal conductance of *Pinus echinata* seedlings was effected by the presence of new root growth. The mean water potential of seedlings with new roots was at least -1.0 MPa higher than the mean of seedlings without new roots and stomatal conductance was also higher where new roots had been produced. Kaushal and Aussenac (1989) reported on large individual plant variation for measurements of photosynthesis and transpiration, which they suggested may be attributed to new root growth. New roots have the potential to increase plant water potential because they increase the root system surface area and, compared to old roots, they occupy wetter soil with less resistance to water movement, have better contact with soil and are more permeable (Brissette and Chambers, 1992).

Divergence in the mass of new roots produced between the control and severe water stress treatment occurred between 18 to 20 days after treatment initiation and corresponded to 10 days of growth where the gravimetric soil water content from around the root plug was below wilting point. This coincided with a decrease in shoot water potential to below -1.5 MPa and the point at which stomatal closure occurred. However, despite the cessation in growth and conductance, the seedlings in the severe water stress treatment were in no way critically stressed such that mortality occurred. Mortality did not occur for the next 12 days, even though shoot water potential declined to -3.0 MPa during this period. The seedlings therefore survived up to 22 days in soil where the water content at the root plug was below wilting point. It is difficult to define the water potential at which a seedling is physiologically dead, and it may dependent on both the duration and intensity of the stress. For this study it was taken to be the water potential at which recovery, subsequent to rewatering, did not occur. This occurred only when shoot water potential declined to below -3.0 MPa. Kaushal and Ausenac (1989) showed that for cedar transplants mortality started to occur below -3.0 MPa and at -5.5 MPa all the plants were dead. For *Pinus nigra* mortality started to occur at a shoot water potential of -2.5 MPa and below -4.0 MPa there was no survival.

This result corroborates what has been observed in field plantings of *P. patula*, where planting with water has generally not increased survival (Rolando and Little, 2007; Rolando and Crous, 2008). Despite the significance of this result, the importance of wet soil at the time of planting should not be under-estimated. In a previous pot trial Rolando and Little (2008; Chapter 5) found that planting *P. patula* seedlings directly into dry soil resulted in mortality occurring soon after planting. Similarly, in a field trial, application of 0.5 litres of water into the planting pit at the time of planting into a dry soil was sufficient to increase survival to a level equivalent to that where 4 litres had been used (Rolando and Little, 2007). The significance of water in establishing root to soil contact has been highlighted with research carried out elsewhere (Nambiar *et al*., 1979).

Divergence in measures of stomatal conductance between the control and severe water stress treatment commenced seven days after treatment initiation when shoot water potential was -0.87 MPa and soil water content was 34% in the severe water stress treatment. Similarly, in a study to investigate the effect of water stress on growth and physiology of *P. patula* seedlings Rolando *et al*. (2008; Chapter 5) found divergence in measures of stomatal conductance between the control and water stress treatments occurred when shoot water potential was -0.8 to -0.9 MPa. Actual stomatal closure in the current trial occurred 21 days after treatment initiation when shoot water potential was between -1.8 and -2.1 MPa. Many investigations have shown that stomata can remain fully open until a critical leaf water potential is reached (Whitehead, 1980). From this value, the aperture begins to narrow with further water loss and closure complete within about 0.5 MPa of the threshold. Hsiao *et al*., (1976) proposed generalised threshold values of -0.5 to -1.0 MPa for mesophytes and -1.0 and -2.0 MPa (and lower) for xerophytes. Although the shoot water potential at which stomatal regulation occurs differs among species and with the degree of adaptation to a habitat (Fitter and Hay, 2002), the values recorded for this study and that collected by Rolando *et al*. (2008; Chapter 5) are consistent with other measures for pine seedlings. The water potential threshold at which stomatal closure occurs in *P. engelmannii* seedlings has been documented as -2.0 MPa (Barton and Teeri, 1993). The threshold of stomatal closure in *P. ponderosa* seedlings, a species considered to be drought tolerant, was reached when water potential values were between -1.65 and -1.73 MPa (Lophushinsky, 1990).

6.5 CONCLUSION

The major findings of this study were; 1) the effect of seedling size on the ability to produce new roots and tolerate water stress, and 2) that in a controlled environment, *P. patula* seedlings were not as sensitive to high air and soil temperatures and low soil water availability as previously expected. Seedlings were able tolerate low soil water availability for several weeks, and were able to recover from moderate to severe water stress. This supports the supposition that planting *P. patula* seedlings with water does not always increase survival. However, this statement must not be confused with the role that moist soil plays in establishing root to soil contact between the root plug and the surrounding soil at planting. Further investigations should focus on the interaction of root plug size and soil water availability on subsequent growth and physiology, the interaction of season of planting with tolerance to water stress and the interaction of water stress with disease resistance for *P. patula* seedlings.

CHAPTER 7

USING A CASH FLOW ANALYSIS TO ESTIMATE THE MINIMUM INCREASE IN SURVIVAL REQUIRED TO RECOVER PLANTING METHOD COSTS FOR A *PINUS PATULA* PULPWOOD ROTATION

ABSTRACT

Using a cash flow analysis, a simple financial model was used to estimate the minimum increase in survival at canopy closure required to recover the planting costs (or break-even value) for a pine pulpwood rotation. To illustrate this, the minimum increase in survival relative to a dry plant was used to estimate the costs that needed to be recovered when; 1) using water or 2) applying an insecticide to the water used in the planting operation. This principle could also be applied to other planting practices, for example the use of a hydrogel, fungicides or fertilisers at planting. Tree volume at harvesting from sites of different quality was also used to illustrate the potential impact of site quality on planting method costs. The data generated by the model were compared to actual research data for water versus dry planting and the use of an insecticide in the planting operation. The value of the approach discussed in this report lies in its simplicity and in the expression of the results in terms of survival, a unit of measure easily understood by foresters. It could also be useful to researchers as a simple tool to compare various silvicultural operations for all species grown in South Africa.

7.1 INTRODUCTION

In terms of forest management, foresters must continuously decide between alternative courses of action, most of which have financial implications. Examples of these include choice of species, different silvicultural practices during regeneration as well different pruning, thinning, harvesting and transport methods. Choosing between different options is simplified if monetary values can be assigned to the alternatives (Uys, 2000). When selecting different methods of planting, the most cost effective practice is to choose the method that gives the best survival at lowest cost, provided this is above a minimum threshold (Morris, 1995). For this, the mean survival data from experimental plots is often used as the decision criterion (Fox, 1998). Traditional "break-even" analysis determines the minimum harvest volume required to earn a specified return on capital invested in forest management activities, such as regeneration, fertilisation or thinning. This minimum required harvest volume can then be compared to regional or localised averages to determine if the proposed investment appears reasonable in terms of physical timber growth (Fox, 1998).

Whole stand growth and development over time, and the influence of tree size distribution, wood quality, site quality, planting density, silviculture, operational costs and timber prices on financial viability and the risk of forestry investments is very dynamic and difficult to define (Kotze, 2006). To understand the potential benefits and risks of different operations and practices, as well as factors that affect their overall effectiveness, it is important to develop tools that illustrate potential outcomes to enable informed decision making. Usually these tools are in the form of integrated models that provide an indication of potential outcomes for different scenarios. However, these tools provide only an indication of what actually occurs in reality and are not deterministic.

This report describes a simple financial model used to estimate the minimum increase in survival required to recover the planting costs (or break-even value) for a pine pulpwood rotation. To illustrate this, the minimum increase in survival relative to a dry plant was used to estimate the costs that needed to be recovered when; 1) using water or 2) applying an insecticide to the water used in the planting operation. This principle could also be applied to other planting practices, for example the use of a hydrogel, fungicides or fertilisers at planting. Tree volume at harvesting from sites of different quality (productivity) was also used to illustrate the potential impact of site quality on planting method costs. In the context of this report, "dry planting" refers to the practice of manually planting seedlings into a planting hole and not adding water during the planting operation and "water planting" refers to the addition of water (usually one to two litres) into the planting hole when planting. The pesticide treatment was included as previous research had shown relatively large increases in survival in response to the inclusion of a pesticide in the water when applied at planting (Crous, 2005; Rolando, 2006). If the use of a pesticide in the water can be shown to be economically viable, this could provide justification for its use during the planting operation. To include the effect of blanking (replacement of dead seedlings) on regeneration costs, the model was developed for three levels of blanking for each planting method and included no blanking, medium intensity blanking (11-30% of dead trees replaced) and high intensity blanking (31-50%).

7.2 DESCRIPTION OF THE MODEL

The profit to be gained from any investment (silvicultural or genetic) in growing trees is affected both directly and indirectly by the method used, as any improvement in yield needs to be taken into consideration together with any monetary input in terms of expenditure (Little *et al.*, 2002). In order to determine this, the cost of an investment needs to be subtracted from its profits (income). It is generally recognised in forestry economic literature that the most acceptable method for assigning values to long-term projects such as forestry is the discounted or compounded cash flow analysis (Uys, 2000). The superiority of this technique and the characteristic which distinguishes it from other techniques is the recognition that money has a "time value" and in forestry the importance of time (in terms of rotation length) cannot be ignored (Uys, 2000). One of the most important decisions associated with such a cash flow analysis is the selection of an appropriate rate of return. Most cash flow criteria are sensitive to changes in the rate of return and consequently small changes in the rate can significantly alter project rankings and profitability calculations (Uys, 2000). The model used in this study was based on a compounded cash flow analysis, calculated for three rates of return (3, 5 and 7%) selected to reflect a range of average real rates of return for short rotation pine plantations in South Africa (Godsmark, *pers. comm.*, 2007[1]). The real rate of return on an investment is the inflation adjusted rate and reflects the reduced purchasing power of the original investment (Klemperer, 1996).

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7.2.1 Growing costs

Costs included in the model are those associated with regeneration and weeding as well as overhead (administration) costs. Total regeneration costs included preparation of the site for planting (harvest residue management and pitting) as well as costs for the planting operation. An estimation of the costs for regeneration (South African rands per hectare, R ha^{-1}), including costs for either dry or water planting or the application of a pesticide in the planting water (0.5 ml $litre^{-1}$), were obtained for the growing region. These costs were structured to account for no blanking, medium intensity blanking (11-30%) and high intensity blanking (31-50%), for dry planting, water planting (1.5 litres) and planting with water (1.5 litres) treated with an insecticide (@ 0.5 ml $litre^{-1}$) (Table 7.1). The estimated total cost of weeding to canopy closure (approximately 3 years) was R 1590.00 ha^{-1} and annual overhead costs were estimated at R 780.00 $ha^{-1}yr^{-1}$ (Forest Economic Services, 2005). The projected future, or compounded value, of all growing costs over 15 years (average age for pine pulpwood rotation) were calculated for the selected real rates of return (3, 5 and 7%) using equations 1 (regeneration and weeding costs) and 2 (overhead costs) (Table 7.1), (Klemperer, 1996).

$$V_n = V_0 (1+i)^n \qquad \textit{Equation 1}$$

$$V_n = a\left(\frac{(1+i)^n - 1}{i}\right) \qquad \textit{Equation 2}$$

where V_o and V_n represent the present and future value of the investment in Rands,
i is the return or interest rate,
a is the amount of the periodic payment and
n is the number of years (length of rotation).

7.2.2 Income

For the cash flow model, the income per hectare (R ha^{-1}) at rotation end was based on total utilisable underbark volume (m^3 ha^{-1}). For illustrative purposes, data from Morris (1995) were used to calculate the total utilisable underbark volume (m^3 ha^{-1}) for a *P. patula* pulpwood stand at 15 years as affected by a range of early stocking reductions (when imposed at canopy closure). These data allowed for the calculation of actual final yield in response to survival ranging from 50 to 95% (Figure 7.1). The average standing price for pine pulpwood (R ton^{-1}) in 2005 was estimated at R 90.00 ton^{-1} (Forest Economic Services,

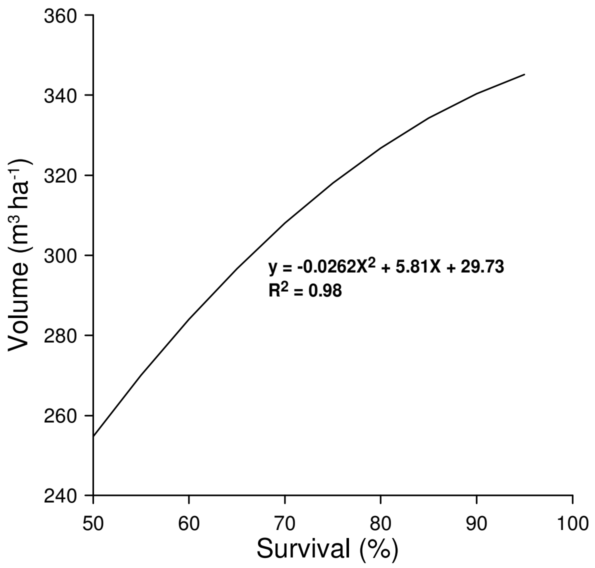

Figure 7.1. Total utilisable volume (m^3 ha^{-1}) of *P. patula* at 15 years in response to an early reduction in survival for a pulpwood stand planted at 1333 stems ha^{-1}. Depending on treatment, a predetermined number of trees were removed at canopy closure to simulate mortality. The values used for this figure were obtained from data collected by Morris (1995).

2005). The standing price reflects the value of the timber less harvesting and transport costs. For this study a conversion ratio of tons to cubic meters of 1.00 m^3 ton^{-1} was used (Forest Economic Services, 2005) and the income per hectare (R ha^{-1}) at rotation end was based on total utilisable underbark volume (m^3 ha^{-1}) calculated for the range of survival values tested (50 to 95%).

7.2.3 Break-even analysis

The return on an investment is calculated as the income (standing value of timber in R ha^{-1} at rotation age) less growing costs (compounded to rotation age in R ha^{-1}) and for this study was calculated for the ranges of survival and regeneration scenarios listed (Table 7.1). For the model, the cheapest method of planting is used as a reference for all subsequent “break-even” calculations. The “break-even” value calculated represents the minimum increase in survival that would be required to recover the costs of a more expensive planting method. For example, the minimum increase in final volume, as affected by an increase in survival, required to recover the cost of the use of water in the planting operation relative to a dry planted operation. A treatment with a positive economic value would thus need to effect an even greater increase in survival (or growth), than the break-even value, to show an increased profit potential. Treatments with a positive

Table 7.1. Estimated costs (R ha^{-1}) for selected regeneration practices including dry planting, water planting (1.5 litres) and the use of an insecticide (0.5 ml $litre^{-1}$) in the water. The costs were adjusted to reflect scenarios where no blanking, medium intensity blanking (11-30%) and high intensity (31-50%) blanking operations were carried out. The adjustment of the costs for the cash flow model are shown below and include weeding and overhead costs.

Activity		Dry plant (R ha^{-1})			Water plant (R ha^{-1})			Insecticide (R ha^{-1})		
		Blanking			Blanking			Blanking		
		None	**11-30%**	**31-50%**	**None**	**11-30%**	**31-50%**	**None**	**11-30%**	**31-50%**
Slash burning		107.50	107.50	107.50	107.50	107.50	107.50	107.50	107.50	107.50
Full cover spray		227.70	227.70	227.70	227.70	227.70	227.70	227.70	227.70	227.70
Manual pitting		500.90	500.90	500.90	500.90	500.90	500.90	500.90	500.90	500.90
Plant cost		600.00	712.50	839.00	600.00	712.50	839.00	600.00	712.50	839.00
Planting (water/dry) @ 1333 stems ha^{-1}		308.50	308.50	308.50	550.50	550.50	550.50	550.50	550.50	550.50
Blanking (water/dry)		0	173.30	251.50	0	314.00	455.80	0	314.00	455.80
Pesticide		0	0	0	0	0	0	104.20	125.60	146.40
Total cost		1744.60	2030.40	2235.20	1986.60	2413.10	2681.40	2090.80	2538.60	2827.70
Compounded cash values at rotation age										
	Total regeneration costs	2718.10	3482.30	3482.30	3095.00	3759.50	4177.50	3257.40	3955.10	4405.50
3%	Weeding	2477.20	2477.20	2477.20	2477.20	2477.20	2477.20	2477.20	2477.20	2477.20
	Overheads	14507.15	14507.15	14507.15	14507.15	14507.15	14507.15	14507.15	14507.15	14507.15
	Total regeneration costs	3626.90	4646.70	4646.70	4129.90	5016.60	5574.40	4346.60	5277.60	5878.70
5%	Weeding	3305.00	3305.00	3305.00	3305.00	3305.00	3305.00	3305.00	3305.00	3305.00
	Overheads	16831.28	16831.28	16831.28	16831.28	16831.28	16831.28	16831.28	16831.28	16831.28
	Total regeneration costs	4813.50	6166.90	6166.90	5481.00	6657.70	7398.00	5768.60	7004.20	7801.80
7%	Weeding	4386.90	4386.90	4386.90	4386.90	4386.90	4386.90	4386.90	4386.90	4386.90
	Overheads	19600.64	19600.64	19600.64	19600.64	19600.64	19600.64	19600.64	19600.64	19600.64

economic value would be the most desirable as the expense associated with implementation would be justified in terms of the additional profits to be gained.

To estimate the break-even value for the range of regeneration scenarios tested in this study, the return on investment when using water or water treated with an insecticide in the planting operation was compared to that for dry planting over all levels of survival, as estimated by the use of Morris (1995) data. An iteration process was used to equate the returns, using the different survival values in the dry planted treatment as a point of reference. This process was repeated for all levels of survival for the different blanking scenarios and rates of return. An example of a spreadsheet has been included in Appendix 7.1.

7.2.4 Application of the financial model

To illustrate the potential applications, the data generated by the model (as described above) were compared to actual research data for dry versus water planting and the use of an insecticide in the planting operation. In addition to the above, the model was also used to estimate the cost implications of different planting methods (dry versus water) for *P. patula* planted on a higher versus lower quality site (using data from Bredenkamp, 1980) (Appendix 7.2). Further details are provided in the relevant sections in the worked examples.

7.2.5 Parameters and assumptions of the model

The use of a simplified model to represent a dynamic reality can be misinterpreted and therefore the parameters and assumptions must be stated. The parameters for this study included; 1) the conditions specific to the yield data (Bredenkamp, 1985; Morris, 1995) in terms of species, planting density, site quality, rotation length and climatic conditions during the rotation, and 2) estimated growing costs, rates of return and standing price for timber used (R ton^{-1}) (Table 7.1). Altering any of the parameters in a model changes the outcome of the analysis. *The growing costs, rates of return and price for timber used are only illustrative for the purposes of this study*. The major assumptions implicit in the model are; 1) that all blanked trees survive and contribute equally to growth, and 2) that mortality due to factors beyond the foresters control (i.e. hail, frost, fire, snow and pest attacks during the post-canopy phase) did not alter the impact of the initial planting treatments.

7.3 WORKED EXAMPLES

7.3.1 Water planting versus dry planting

There is currently little evidence that planting *P. patula* seedlings with water increases growth (in the short term and long term) over those that are dry planted (Rolando and Little, 2007), therefore any increase in final yield in response to the use of water in the planting operation is most likely as a result of an increase in survival. As the rate of return is implicit to the cash flow model, it is expected that greater increases in survival would be required to recover the costs of planting with water when capital is invested at higher rates of return (Table 7.2). In addition, the greatest percentage increase in survival required to recover planting method costs will occur in response to the scenario with greatest initial input at regeneration, as for the high intensity blanking in this study (Table 7.2). Where survival is good (above 85%), it becomes less possible to recover the actual planting costs for some of the more costly scenarios. This occurs due to the asymptotic shape of the curve in the yield model, where there is a decline in the rate of volume increase as the target density (stocking) is attained (Figure 7.1).

Table 7.2. Estimated increase in survival required to recover planting method costs using a cash flow analysis (for three rates of return: 3, 5 and 7%), calculated for yield data from a *P. patula* pulpwood stand planted at 1333 stems ha^{-1} (Morris, 1995). The reference survival is that for a dry planted operation of equivalent blanking intensity. Values represent the projected minimum increase in survival required to recover the cost of planting with water or the application of an insecticide to the water. Shaded cells indicate scenarios where the cost could not be recovered.

Reference survival of a dry plant (%)	Increase in survival (%) required to recover the costs of water planting								
	No blanking			Blank 11-30%			Blank 31-50%		
	3%	5%	7%	3%	5%	7%	3%	5%	7%
50	1.5	1.8	2.6	2.4	3.0	3.9	2.5	3.5	4.6
55	1.5	1.8	2.6	2.3	3.0	3.9	2.5	3.5	4.6
60	1.5	1.9	2.7	2.5	3.1	4.2	2.6	3.8	4.9
65	1.6	2.2	3.1	2.8	3.5	4.8	3.0	4.4	5.7
70	1.9	2.6	3.6	3.3	4.2	5.8	3.6	5.1	6.9
75	2.4	3.1	4.4	4.0	5.2	7.0	4.4	6.2	8.5
80	3.0	3.8	5.5	4.9	6.6	8.5	5.4	7.5	10.5
85	3.8	4.7	6.7	6.0	8.3	10.4	6.7	9.0	12.9
90	4.8	5.7	8.2	7.3	10.2	12.5	8.3	10.8	15.8
	Increase in survival (%) required to recover the cost of an insecticide								
50	2.1	2.7	3.6	2.9	4.1	5.2	3.4	4.7	6.3
55	2.1	2.7	3.7	2.9	4.2	5.5	3.4	4.7	6.5
60	2.2	3.0	4.0	3.2	4.5	6.0	3.7	5.1	7.2
65	2.5	3.4	4.5	3.6	5.2	6.9	4.2	5.8	8.3
70	3.0	3.9	5.4	4.2	6.1	8.2	5.0	7.0	9.9
75	3.6	4.7	6.4	5.0	7.4	9.8	6.0	8.6	12.0
80	4.4	5.6	7.7	6.1	9.0	11.7	7.2	10.7	14.5
85	5.4	6.6	9.3	7.3	10.9	13.9	8.7	13.2	17.5
90	6.5	7.9	11.1	8.7	13.2	16.5	10.4	16.1	21.0

As already stated, the data projected by the model needs to be compared to actual plantation survival records or trial data for it to have any practical value. To do this, data from 58 *P. patula* research trials that included a dry planted and a water planted treatment were used as a point of reference (Rolando and Crous, 2008; Appendix 7.3). No blanking was carried out in these trials. Over all 58 records, average survival for the water planted (83.7%) relative to dry planted (80.3%) treatments resulted in an average increase of 3.4%. Assuming these trials were planted on sites of higher quality at 1333 stems ha^{-1}, the use of water in the planting operation would have provided a sufficient increase in survival to recover the costs of the operation only at the lowest rate of return (Table 7.2). However, there is no evidence of an overall positive economic value (or increased profit potential), associated with the more expensive planting method (water planting in this example).

An alternative approach to benchmark the data projected by the model would be to calculate the minimum increase in survival required to break-even on an individual trial basis to determine the number of occasions where planting with water achieved at least a break-even level of survival. Based on a 5% real rate of return, and including only trials with a minimum survival of 50% (Appendix 7.3), only 12 out of 50 trials (or 24% of the trials) showed the increase in survival in the water planted treatment to be sufficiently large to recover the costs of the planting method (provided the difference in survival carried through to felling).

7.3.2 Application of an insecticide to the water

Using data collected from 31 research trials, Rolando (2006) showed an average increase in survival (over a water pant) during regeneration of 4, 14 and 9% where an insecticide, fungicide or both were applied in the water at planting. As with water planting, there is currently little evidence that planting *P. patula* seedlings with an insecticide or fungicide increases long term growth over those dry planted (Rolando, 2006). Any affect on final yield in response to the use of a pesticide in the water at planting is therefore likely to be as a result of an increase in survival. Because of the cost of the insecticide, even greater increases in survival over a dry planting are required by the model to recover the planting method costs (Table 7.2).

For the 31 trials, comparisons were made relative to survival in a water planted control and not to a dry plant as previously discussed, as insecticide is only applied in water (Rolando, 2006). As an example, data from the 31 trials using insecticide in the water were compared to values predicted by the model. The model was therefore reconstructed to reference the insecticide treatment relative to a water plant (the reference treatment) (Table 7.3). No blanking was carried out in any of the trials. Over all 31 trial records, average survival for the water and insecticide treatment (84.7%) relative to the water planted treatment (80.7%) resulted in an average increase of 4.1%. Assuming these trials were planted on higher quality sites at 1333 stems ha^{-1}, the addition of an insecticide to the water would have provided a sufficient increase in survival to recover the costs of the insecticide (Table 7.3).

The minimum increase in survival required to break-even with a water planted treatment was calculated for a return rate of 5%, and compared to the research data on an individual trial basis (Appendix 7.4). Sixteen of the 31 trials (52%) showed an increase in survival relative to the water planted treatment sufficiently large to recover the costs of the insecticide (provided the difference in survival carried through to felling).

Table 7.3. Estimated increase in survival required to recover planting method costs using a cash flow analysis (for three rates of return: 3, 5 and 7%) for yield data from a *P. patula* pulpwood stand planted at 1333 stems ha^{-1} (Morris, 1995). The reference survival is that for a water planted operation of equivalent blanking intensity. Values represent the projected minimum increase in survival required to recover the cost of planting with the application of an insecticide to the water.

Reference survival	No blanking		
of a water plant (%)	3%	5%	7%
50	0.7	0.8	1.1
55	0.7	0.9	1.1
60	0.8	1.0	1.2
65	0.9	1.1	1.3
70	1.0	1.3	1.5
75	1.2	1.5	1.8
80	1.4	1.7	2.1
85	1.7	2.0	2.5
90	2.0	2.3	3.0

7.3.3 Planting method cost implications associated with site quality (higher versus lower quality site)

In short rotation pulpwood crops, stand density has a stronger influence on yield on higher quality rather than lower quality sites. This is due to a greater percentage decrease in yield in response to a reduction in optimum stocking on good quality (higher yield) relative to poor quality (lower yield) sites (Bredenkamp, 1980; Kassier and Kotze, 2000). Any model predicting the effects of mortality, or lowered stocking, on final yield and corresponding cost implications, should consider these effects as the cost implications may be different for higher versus lower quality sites. To provide an indication of the effect of site quality on cost implications for different planting methods (water planting versus dry planting) using the cash flow model, mensuration data representing the impact of stocking on final yield for a higher versus lower quality site were tested (Bredenkamp, 1980; Appendix 7.2). The yield data were adjusted to reflect 1333 stems ha^{-1} as full stocking (or 100% survival), and yield for planting densities below this were used to reflect the impact of reduced survival on yield relative to 1333 stems ha^{-1} (Figure 7.2).

The minimum increase in survival required to recover planting with water on an higher versus lower quality site was calculated using the cash flow model, for a return rate of 5% (Table 7.4). While it is difficult to relate the projected figures to actual data, the importance of these data lie in the greater percentage increase in survival required to recover the cost of using water in the planting operation on a lower versus higher quality site. For example, a 6% versus 2.8% increase in survival is projected to recover the costs of using water for a lower versus higher quality site as referenced to a survival of 70% in a dry planted operation (Table 7.4).

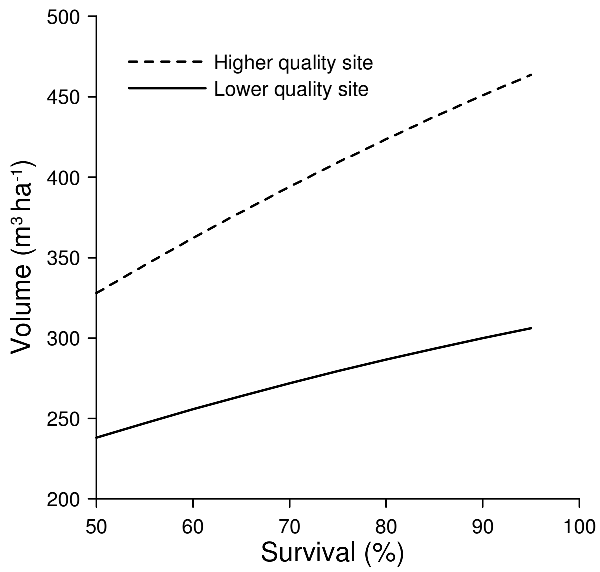

Figure 7.2. Total utilisable volume ($m^3\,ha^{-1}$) of *P. patula* at 16 years for a higher and lower quality pulpwood stand obtained from mensuration data collected by Bredenkamp (1980). The data were adjusted to reflect 1333 stems ha^{-1} as full stocking (or 100% survival), with yield for planting densities below this used to reflect the impact of reduced survival on yield relative to 1333 stems ha^{-1}.

Table 7.4. Estimated increase in survival required to recover planting method costs using a cash flow analysis (calculated for a return rate of 5%), for yield data from a higher versus lower quality *P. patula* site (Bredenkamp, 1980). The reference survival is that for a dry planting operation of equivalent blanking intensity. Values represent the minimum increase in survival required to recover the costs of planting with water.

Reference survival of a dry plant (%)	No blanking		Blank 11-30%		Blank 31-50%	
	Site quality		Site quality		Site quality	
	Higher	Lower	Higher	Lower	Higher	Lower
50	1.6	3.2	2.5	5.0	3.0	5.8
55	1.7	3.3	2.5	5.2	3.2	6.1
60	1.7	3.5	2.6	5.4	3.3	6.4
65	1.8	3.7	2.7	5.7	3.5	6.7
70	1.9	3.9	2.8	6.0	3.6	7.1
75	1.9	4.1	2.9	6.4	3.8	7.4
80	2.0	4.4	3.0	6.7	4.0	7.8
85	2.1	4.6	3.2	7.1	4.2	8.3
90	2.2	4.9	3.3	7.5	4.4	8.7

Intuitively, it is on the lower quality sites where foresters may want to apply more costly regeneration methods to increase initial survival. The projected break-even survival figures are specific for the data used in this example and would change relative to changes in yield, return rate and regeneration costs. The importance of this example lies in the illustration of the interaction of site quality with projected planting method costs.

7.4 CONCLUSION

Although based on tree growth data from research trials, all data presented in this report are used to illustrate a principle and cannot be used to justify the utilisation of any planting methods. Individual companies or forest owners would need to provide relevant yield and economic data for their own landholdings to project appropriate break-even survival values. These values would need to be compared to regional or localized averages to determine if the proposed investment appeared reasonable in terms of actual timber growth and survival. The value of the approach discussed in this report lies in its simplicity and in the fact that the results of the analysis are expressed in terms of survival, a unit of measure easily understood by foresters. It could also be useful to researchers as a simple tool to compare various silvicultural operations for all species grown in South Africa.

It is also important to note that the economics of using a particular treatment at planting is dependent on the frequency at which a positive response in terms of survival and/or growth is achieved. In other words, there is always an element of risk that the expected return will not be achieved. Rolando and Crous (2008) and Rolando *et al*. (2006) showed that the potential for an increase in survival in response to planting with water was most likely to occur when planting during (late) winter and spring, when soil water availability is low or rainfall sporadic. The likelihood of recovering the extra costs of planting with water (or reaching a break-even point) is therefore higher during these periods. In contrast, Rolando (2006) showed that the inclusion of an insecticide in the water at planting was most likely to increase survival when used during summer and autumn.

Most commercial timber inventories estimate stand density at a 5-10% error rate, therefore it may not be practically possible to detect small increases in survival (2-10%) in response to different planting methods. This would make an evaluation of the success of different planting methods very difficult, but may also indicate a negligible cost:benefit situation. When examining the cost implications of different planting methods, some consideration should also be given to the need to procure additional pulpwood to meet demand at the mill. As long as market pulpwood prices exceed growing costs, the need to supply a market demand that exceeds the production capacity, will favour regeneration methods that contribute to higher yields regardless of the cost.

APPENDIX 7.1

Spreadsheet for cash flow model using data from Morris (1995) and estimated costs for dry and water planting
Shaded cells indicate parameters that can change and are shown here for example only

Rotation Information	Amount	Planting method Costs	
		A[#]	B[##]
Return rate (%)	3		
Rotation length (years)	15		
Annual overhead costs R ha^{-1}	R 780.00		
Total establishment costs R ha^{-1}		R 1744.00	R 1986.60 (See Table 1)
Weeding to canopy R ha^{-1}	R 1590.00		
Standing Value (R m^{-3})	R 90.00		

([#]Dry plant:No blank) ([##]Water plant:No blank)

Planting method A
Dry plant: No Blank

Survival (%)	Yield (m^3 ha^{-1})	Income	Compounded costs (R ha^{-1}): Establishment	Weeding	Annual overheads	Net Value
50	254.71	R 22923.90	R 2717.10	R 2477.17	R 14507.15	R 3222.48
60	283.99	R 25559.10	R 2717.10	R 2477.17	R 14507.15	R 5857.68
70	308.03	R 27722.70	R 2717.10	R 2477.17	R 14507.15	R 8021.28
80	326.82	R 29413.80	R 2717.10	R 2477.17	R 14507.15	R 9712.38
90	340.38	R 30634.20	R 2717.10	R 2477.17	R 14507.15	R 10932.78

BREAK EVEN CALCULATIONS

Survival (X)	Value (Y)[#]	Break even survival (% increase)
51.3	R 3213.74	1.3
61.6	R 5857.49	1.6
72.0	R 8019.14	2.0
82.7	R 9710.64	2.7
94.3	R 10934.30	4.3

[#] $y = -2.3586x^2 + 522.96x - 17407$

Planting method B
Water plant: No blank

Survival (%)	Yield (m^3 ha^{-1})	Income	Compounded costs (R ha^{-1}): Establishment	Weeding	Annual overheads	Net Value
50	254.71	R 22923.90	R 3095.06	R 2477.17	R 14507.15	R 2844.52
60	283.99	R 25559.10	R 3095.06	R 2477.17	R 14507.15	R 5479.72
70	308.03	R 27722.70	R 3095.06	R 2477.17	R 14507.15	R 7643.32
80	326.82	R 29413.80	R 3095.06	R 2477.17	R 14507.15	R 9334.42
90	340.38	R 30634.20	R 3095.06	R 2477.17	R 14507.15	R 10554.82

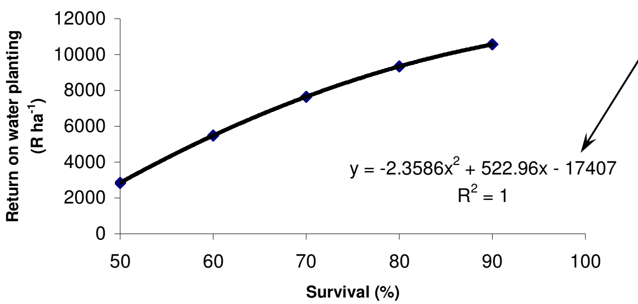

Figure 1. Estimated return on a water planted operation for the different levels of survival

APPENDIX 7.2

The data used for the estimation of total utilisable underbark volume (m^3 ha^{-1}) for *P. patula* grown for pulpwood on two different quality sites were obtained from Bredenkamp (1980), (**Figure A**). These two sites formed part of the pine Correlated Curve Trend (CCT) trial series and were located at Nelshoogte (Trial series A) and Weza (Trial No. 522/4), (Bredenkamp, 1980; von Gadow, 1983). These sites were classified as site quality II+ and III+, with mean heights at age 20 years of 21.2 m and 18.1 m respectively (von Gadow, 1983). The quality of the growing site is traditionally expressed by the site index, i.e. the top height attained at a particular (index) age (von Gadow, 1983). The top height is a useful criterion as it is not affected by thinnings. Mean height, though not as popular, can also be used to describe the quality of a growing site and for these sites the published site index referred to the mean height attained at age 20 (von Gadow, 1983). Based on the site index calculated for these sites (von Gadow, 1983), they are equivalent to that described by Kassier and Kotze (2000) as average (Nelshoogte) and poor (Weza) sites for *P. patula* pulpwood. For convenience the trials are referred to as the higher and lower quality sites in the report.

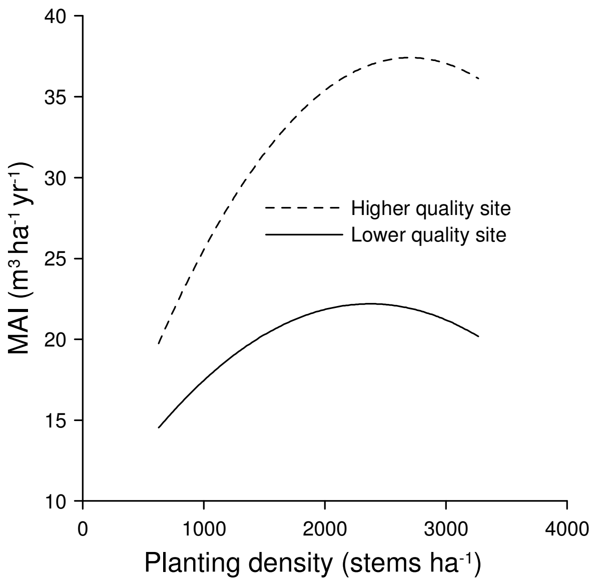

Figure A. The influence of planting density on mean annual increment (m^3 ha^{-1} yr^{-1}) at age 16 years in unthinned treatments for *P. patula* CCT trials located at Nelshoogte (Trial series A; higher quality site) and Weza (Trial No. 522/4; lower quality site) (Bredenkamp, 1980; von Gadow, 1983).

APPENDIX 7.3

Three months survival (%) data from 58 *P. patula* trials in response to dry and water planting. The cash flow model (CFM), calculated for a return rate of 5%, was applied to the data. If the actual difference in survival between treatments was greater than the percentage increase required by the cash flow model to break-even (i.e. cover costs of using water), that trial successfully met the requirements. No blanking was carried out. Shaded cells had a minimum survival below 50%, which fell outside the regression upon which the model is based (Figure 7.1).

	Survival (%)		Actual difference	CFM (5%)	# Meet
	Dry plant	Water plant	(%)	% increase	requirements
	12.1	17.6	5.5	7.2	na
	13.0	80.0	67.0	7.0	na
	39.8	62.5	22.7	2.5	na
	43.8	31.0	-12.8	2.2	na
	45.5	44.0	-1.6	2.1	na
	46.5	55.6	9.1	2.0	na
	47.0	87.0	40.0	2.0	na
	49.0	84.0	35.0	1.9	na
	52.0	58.0	6.0	1.9	1
	56.6	61.3	4.7	1.9	1
	56.7	57.5	0.8	1.9	0
	59.8	47.0	-12.8	1.9	0
	61.6	54.6	-7.0	2.0	0
	66.3	63.6	-1.7	2.2	0
	72.6	60.7	-11.9	2.8	0
	73.4	85.5	12.2	2.9	1
	75.9	83.3	7.4	3.2	1
	77.0	86.0	9.0	3.4	1
	78.0	73.0	-5.0	3.5	0
	78.8	75.7	-3.1	3.6	0
	81.5	88.9	7.4	4.1	1
	82.9	90.4	7.5	4.3	1
	83.0	97.0	14.0	4.3	1
	83.0	94.0	11.0	4.3	1
	83.0	85.0	0.5	4.6	0
	84.4	93.6	8.0	4.8	1
	85.6	90.7	3.7	5.0	0
	87.0	95.0	7.5	5.1	1
	87.5	89.8	2.3	5.1	0
	87.5	88.9	0.0	5.4	0
	88.9	95.0	4.0	5.9	0
	91.0	96.0	5.0	5.9	0
	91.0	92.5	0.5	6.1	0
	92.0	87.0	-5.0	6.1	0
	92.0	99.4	7.3	6.1	1
	92.1	94.0	0.0	6.6	0
	94.0	88.9	-5.5	6.7	0
	94.4	92.6	-1.8	6.7	0
	94.4	95.0	0.0	6.8	0
	95.0	96.0	1.0	6.8	0
	95.0	97.0	1.5	6.9	0
	95.5	99.0	3.0	7.0	0
	96.0	98.0	2.0	7.0	0
	98.0	99.0	1.0	7.6	0
	98.0	98.0	0.0	7.6	0
	98.0	92.0	-6.0	7.6	0
	98.0	98.0	0.0	7.6	0
	98.0	90.0	-8.0	7.6	0
	99.0	99.0	0.0	7.8	0
	99.0	93.5	-5.5	7.8	0
	99.0	100.0	1.0	7.8	0
	99.0	99.0	0.0	7.8	0
	99.0	99.0	0.0	7.8	0
	99.0	96.0	-3.0	7.8	0
	100.0	92.0	-8.0	8.1	0
	100.0	98.0	-2.0	8.1	0
	100.0	99.0	-1.0	8.1	0
	100.0	89.0	-11.0	8.1	0
Mean	**80.3**	**83.7**	**3.4**		**12**

1 = yes; 0 = no

APPENDIX 7.4

Survival (%) data from 31 *P. patula* research trials in response to water planting and planting with an insecticide applied into the water. The cash flow model (CFM), calculated for a return rate of 5%, was applied to the trial data. If the actual difference in survival between treatments was greater than the percentage increase required by the cash flow model to break-even (i.e. recover costs of using insecticides), the trial successfully met the requirements. As these were research trials no blanking was carried out.

	Survival (%)		**Actual difference (%)**	**CFM (5%)**	**# Meet requirements**
	Water plant	**Water plant with insecticide**		**% increase**	
	89.9	90.4	0.5	2.3	0
	96.3	91.3	-5	2.8	0
	45.4	52.4	7	0.8	1
	92.0	93.4	1.4	2.5	0
	85.7	91.3	5.6	2.1	1
	88.9	61.1	-27.8	2.2	0
	83.3	77.8	-5.5	1.9	0
	88.9	83.3	-5.6	2.2	0
	83.3	88.9	5.6	1.9	1
	77.8	94.4	16.6	1.6	1
	94.4	88.9	-5.5	2.6	0
	77.8	77.8	0	1.6	0
	77.8	88.9	11.1	1.6	1
	72.2	100	27.8	1.4	1
	94.4	94.4	0	2.6	0
	72.2	100	27.8	1.4	1
	83.3	100	16.7	1.9	1
	36.3	51.6	15.3	0.8	1
	77.3	56.2	-21.1	1.6	0
	94.5	85.9	-8.6	2.6	0
	52.7	85.9	33.2	0.9	1
	73.8	64.1	-9.7	1.5	0
	55.5	54.7	-0.8	0.9	0
	91.9	96.9	5	2.4	1
	86.6	91.1	4.5	2.1	1
	96.5	98.9	2.4	2.8	0
	93.3	99.8	6.5	2.5	1
	91.1	96.5	5.4	2.4	1
	88.9	93.3	4.4	2.2	1
	89.8	88.9	-0.9	2.3	0
	69.8	89.3	19.5	1.3	1
Mean	**80.7**	**84.8**	**4.1**		**16**

1 = yes; 0 = no

CHAPTER 8

FINAL DISCUSSION AND CONCLUSIONS

8.1 COMMERCIAL FORESTRY AND ITS RELATION TO RAINFALL IN SOUTH AFRICA

It is recognized that considerable temporal and spatial variation in climate, particularly in rainfall, occurs in southern Africa (Tyson, 1986). Coupled with considerable spatial and temporal variation, rainfall is also highly seasonal over most of southern Africa, and is mostly a summer phenomenon in the eastern and northern regions (forestry growing areas) where 80% of the annual rainfall occurs between October and March (Tyson, 1986). According to Shulze (1997) rainfall variability influences agricultural productivity to a greater extent than rainfall magnitude and a general rule of thumb dictates that the higher the mean annual precipitation (MAP) in an area, the lower its year to year variability (Shulze, 1997).

South Africa is a water-scarce country, and water, in terms of mean annual precipitation, has always been the most limiting factor in forestry expansion (Schulze and Kunz, 1995; Versfeld, 1996). High rainfall areas (regions with a mean annual precipitation above 800 mm) that are best suited to forestry are mostly afforested already and there is a move to conserve the remaining montane grasslands, fynbos and forests in these high rainfall areas (Dye, 2000). New afforestation is unlikely to occur and if so, will take place only in the drier areas considered marginal for forestry. The risk of drought in these areas will be relatively high as a result of lower and more variable rainfall (lower MAP), and higher evaporative demand.

For the South African forestry industry, most of the studies on the impacts and risks posed by drought, changes in climate and (or) soil water availability on plantation productivity have been focused on mature trees (Kunz and Smith., 2001; Dye, 2001; Esprey, 2001; Campion *et al*., 2005). Few, if any, investigations have been concerned with the potential impacts of these factors on mortality and growth during regeneration. This is no doubt because the loss in timber in newly planted areas is considerably lower, but, never-the-less, financial losses due to regeneration failure can be substantial.

8.2 THE ROLE OF WATER DURING REGENERATION WITH *PINUS PATULA*

The results from this research have provided an indication of the affect of soil water availability, including the interaction between rainfall, evaporative demand and seedling quality, on early survival, growth and physiology of *P. patula*. A comprehensive understanding of the role of water during regeneration of *P. patula* from a range of perspectives, including applied, basic and economic, has been obtained. It remains to contextualize these results in manner that highlights the integrated nature of the research.

Statistics on rainfall for southern Africa highlight its spatial, temporal and seasonal unpredictability (Tyson, 1986). It is the unpredictable nature of rainfall that has often persuaded commercial forestry companies to include water planting as a "best-operating-practice" during regeneration of *P. patula*. The 'common sense' inherent in planting trees with water is an example of the development and perpetuation of practices based on perceived benefits rather than scientific investigation. It is also likely that the extra cost of using water in the planting operation has been a more frequently applied decision criterion for not using water during planting, rather than a decision based on survival data (there being a lack of robust data on which to base this decision). The results from the applied field trials and the retrospective study conducted for this study (Chapters 2 and 3) indicated that the practice of using water in the planting operation was likely to increase survival of *P. patula* seedlings only when planting during spring and early autumn (there being a lack of data to adequately assess the impact of water used in late autumn and winter planting). These are periods within the rainy season (October to March) when the variability of rainfall is higher than in mid-summer, the peak rainy season for the summer rainfall region (Schulze, 1997). When necessary, a minimum quantity of 0.5 litres of water was shown to be sufficient to increase soil water availability such that survival was unaffected by any lack of rain (Chapter 2). These results highlighted three main factors:

1. Contrary to common practice, planting with water did not generally result in an increase in survival of *P. patula* seedlings following planting. This highlighted the lack of understanding of the ecophysiology of *P. patula* seedlings as well as the

importance of developing an understanding of the cost:benefit implications of planting methods.

2. When necessary, the function of the water was to increase the availability of moisture in the zone immediately surrounding the seedling as well as to increase root to soil contact in this zone. The importance of water in establishing root to soil contact during regeneration has been shown in other forestry growing regions (Nambiar *et al*., 1979). The rate of root elongation measured in the pot trial conducted in 2007 (Chapter 6) showed that the average length of roots three weeks after planting was 4.6 ±0.02 cm. This suggests that seedlings are dependent on soil water availability in the zone immediately surrounding the root plug, and not in the entire pit, for at least one month after planting. Supplying extra water at the time of planting to wet the entire pit (i.e. using more than one litre of water) cannot supplement the water requirements of the seedlings one month after planting, as this water will have either evaporated or drained from the pit when penetrated by new root growth.
3. There was no effect of water applied at planting on early tree growth.

Three pot trials were subsequently conducted to increase the understanding of the physiology of *P. patula* seedlings (Chapters 4-6). Besides providing at indication of the physiological data suited for our purposes, the first pot trial (Chapter 4) highlighted the importance of root plug moisture at the time of planting to subsequent survival. This result is directly applicable to field transport and handling of seedlings in the forestry industry, where poor supervision can result in insufficient wetting of root plugs prior to planting. Simply ensuring wet root plugs at the time of planting can extend the period during which seedlings can survive low soil water availability (Chapter 4). The subsequent two pot trials (Chapters 5 and 6) were aimed at investigating the interaction between planting stock quality (as determined by measures of size) and soil water availability and the effect on survival, growth and physiology of *P. patula* seedlings. The results from these pot trials indicated that:

1. *P. patula* seedlings were not as sensitive to high air and soil temperatures (above 30˚C) and low soil water availability (below -1.5 MPa) as had been previously suspected. On the contrary, the seedlings were able to tolerate low soil water availability for several weeks, and were able to recover from moderate to severe water stress (regarded as a shoot water potential below -1.5 MPa). This data

supported the results from the applied field trials and retrospective studies where the application of water to the seedlings at planting did not substantially increase survival.

2. The critical water potential threshold for changes in stomatal conductance (narrowing of the aperture) was in the region of -0.8 to -0.9 MPa. Stomatal closure for newly transplanted *P. patula* seedlings occurred at a shoot water potential between -1.2 MPa to -1.5 MPa. Mortality due to drought stress is likely to occur only in response to extended periods of low soil water and is associated with a shoot water potential of below -3.0 MPa.
3. There was variability between seedlings in their potential for survival and growth. Bigger seedlings had a greater capacity for new root growth following planting. New root growth, as well as a greater mass of new roots, was associated with higher shoot water potentials and higher rates of transpiration under conditions of low soil water availability. This indicated that seedling quality, as determined by size, may play a role in sensitivity to stress.

The field trials, retrospective study and pot trials indicated that the practice of planting with water was not critical to the survival of *P. patula* seedlings, largely as the seedlings were more tolerant to low soil water availability than previously expected. It may be that other factors such as disease, poor handling between the nursery and field, and seedling quality interact with water stress in field to affect mortality in commercial operations. Research to quantify some of these factors is suggested.

With an understanding of the nature of rainfall in South Africa, an estimation of the effect of water planting on survival, and an understanding of the sensitivity of *P. patula* to water stress, it remained to investigate the cost effectiveness of planting with water, given certain growth parameters and management scenarios. To this end a simple financial model was developed (Chapter 7). To illustrate potential applications, the modelled data were compared to actual research data for water versus dry planting (and the inclusion of an insecticide in the water). While these comparisons were specific to the parameters included in the model for this study, as well as the results of the research trials used in the benchmarking exercises, several important aspects were highlighted:

1. Costs for planting with water were likely to be recovered only when no blanking was carried out, with capital invested at a low return rate (3%). Including an

insecticide in the water increased the likelihood of breaking-even and recovering the costs for using both the water and insecticide.

2. Site quality had an impact on the increase in survival required to recover planting method costs, with a greater percentage increase in survival or yield required on lower quality sites. Lower quality sites often have a lower MAP (associated with higher rainfall variability), or shallow soils (associated with lower soil water availability) and therefore are also likely to be sites where foresters may intuitively want use water to reduce (drought related) mortality. The impact of site quality is thus also an important factor to include in any decisions regarding planting methods and their costs. It is possible that the need for timber is such that these extra costs are negligible, or alternative methods of increasing survival, such as increasing initial stand density, should be examined.
3. Data from the 58 research trials indicated that the seasons with the greatest probability of getting a positive survival response to water planting were spring, autumn and winter and therefore the seasons most likely to provide a return on investment.

8.3 SUMMARY OF KEY OUTPUTS FROM THIS RESEARCH

In summary, the investigations conducted for this thesis have:

1. Established benchmark physiological data for *P. patula* during regeneration for commercial forestry in South Africa.

2.a. Illustrated that planting with water does not always increase survival of *P. patula* seedlings. Only during periods of low soil water availability, for example when planting during early spring and autumn, is there potential for a positive response.

b. Indicated that, when necessary, a minimum quantity of 0.5 litres of water was sufficient to increase soil water availability in the zone of the root plug such that *P. patula* seedling survival was unaffected by any lack of rain.

c. Shown that planting with water has no substantial effect on growth of *P. patula* seedlings planted in the field.

d. Highlighted the importance of a wet root plug at the time of planting to subsequent survival after planting.

3. a. Indicated no significant differences between two *P. patula* families in early growth and physiology in response to soil water availability following planting.

b. Indicated that the critical water potential threshold for changes in stomatal conductance for *P. patula* seedlings was in the region of -0.8 MPa to -0.9 MPa and stomatal closure occurred at a shoot water potential of between -1.8 to -2.1 MPa.

c. Shown that mortality of *P. patula* seedlings occurred when shoot water potential declined to below -3.0 MPa.

d. Illustrated the effect of increased seedling size on the ability to produce new roots and tolerate water stress.

4. Provided an indication of the cost-effectiveness of using water, or water and an insecticide, at planting, for a range of sites and management scenarios.

8.4 FURTHER RESEARCH

Scientists continue to debate the exact mechanisms, impacts and magnitude of climate change, but are now virtually unanimous that the climate is changing and will continue to do so for at least the next century (IPPC, 2001). For southern Africa, regional models of climate change predict an approximate 2˚C rise in temperature and either a 10-15% decrease in summer rainfall in eastern regions, or an increase of severity of drought and storms (Shulze and Kunz, 1995; Joubert and Hewitson, 1997). These projected climate changes, are predicted to affect areas currently suitable to forestry and may require future species and genotype shifts to accommodate growth within the expected climatic changes (Schulze and Kunz, 1995). Changes in rainfall seasonality, humidity conditions and temperature regimes could create conditions which trigger outbreaks of pests and favour their spread (Van Staden *et al*., 2004). Walker *et al.* (1989) stated that this secondary affect of climate change could be of more significance than the primary effects of changes in temperature and rainfall. With these predictions in mind, the role of pest management during regeneration may become increasingly important, particularly for apparently disease susceptible species such as *P. patula.* The application of water treated with an insecticide to the seedling at planting may be a method by which improved pest management strategies can be considered. Since results from studies investigating the potential increase in survival from the use of pesticides at planting are largely favourable (Crous, 2005; Rolando, 2006), further research aimed at identifying suitable products and methods of application during regeneration may be warranted.

The results of this research have shown that while water plays a role in the early survival and growth of *P. patula* seedlings, this factor is not a principle determinant of mortality. This raises questions as to other potential causes of the high mortality observed during regeneration with *P. patula*. Infection of the seedlings with the pathogen *F. circinatum* is currently perceived as one of the greatest threats to the success of regeneration with *P. patula* (seedlings and cuttings) (Coutinho *et al*., 2007). The perceived negative impact of this pathogen on the commercial regeneration of *P. patula* is such that alternative species and hybrids are being investigated for future softwood supply. There is a poor understanding about the mechanisms of infection with the pathogen during regeneration, as well as the environmental stresses that induce its outbreak. This could potentially result in the development of disease management strategies based on 'common sense' rather than scientific investigation. Environmental conditions are known to influence both the severity and incidence of woody plant diseases and water stress has been associated with the enhancement of infection with disease of tree seedlings by many pathogens (Schoeneweiss, 1981; Blodgett *et al*., 1997). Further research should therefore be aimed at investigating the interaction of water stress and infection with *F. circinatum* in *P. patula* seedlings.

The results from the final pot trial (Chapter 6) indicated that seedling size and morphology at planting played a role in subsequent root growth and water stress tolerance. In this regard, further investigations should aim to examine the interaction of root plug size (as determined by container type) and soil water availability on subsequent growth and physiology and the interaction of season of planting with tolerance to water stress. These studies should also aim to examine methods of grading seedlings within a population to select those that have a high potential for survival and growth. It is also suggested that further investigations aim to examine the effects of soil water availability on physiology, survival and growth of *P. patula* cuttings, as well as other pine species and hybrids grown in South Africa, such as *P. elliottii*, *P. elliottii* x *P. caribaea* and *P. patula* x *P. tecunumanii*. The morphology and physiology of pine cuttings differs from that of seedlings and may affect their response to imposed water stresses. Currently cuttings are not commercially deployed on a large scale but this is likely to change in the future.

Roberts (1994) stated that the '…state of knowledge about the spatial and temporal variation in the supply/demand relationships concerning soil moisture status in our plantations is sadly at a very elementary level. There are few moisture profiles that have been monitored in plantations concurrently with measures of tree stress levels and growth rates, that permits an adequate evaluation of temporal dynamics'. While some intensively monitored process trials have been implemented since 1994 (du Toit and Dovey, 2005; Campion *et al*., 2005;), the value of long term, basic research is still not fully recognized by the South African forestry industry. It is suggested that one of the major gaps in basic research is actual data for the development of models that can link rainfall, temperature, soil type, survival and growth for various commercial forestry species at all stages of development, but particularly during the regeneration phase. A greater understanding of the processes driving survival and growth during regeneration could be gained through the implementation of intensively monitored process trials.

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