Chapter Four

Field Trials with Paclobutrazol

4.1 Introduction

In Chapter 3, preliminary experiments were conducted with PAC to establish a rapid, effective and inexpensive method for its isolation and detection in plants. No single method was discovered which could fulfil these criteria. The main difficulties were that PAC is colourless (making visual detection impossible) and, relatively non-reactive (making conversion of the compound to a visible product impossible). Most experimentation with PAC has relied on observation of its effects on plants rather than dealing with its actual mechanism of effect and subsequent isolation and detection. This chapter presents a case study using PAC in a field trial of its effectiveness as a growth retardant on three street tree species.

The first section of this chapter will review the methods of delivery of PAC to plants ranging from soil drenches to foliar sprays and trunk injection. Each method will be examined and evaluated in light of current literature. The second section of the chapter will present the field trial, its results, and the implication of those results for future trials using PAC. Results presented in this chapter from other sources almost exclusively refer to retardation of shoot growth. For a fuller discussion of other effects caused by PAC see Chapter 2.

4.2 PAC Application Techniques

4.2.1 Soil application

The earliest formulation of PAC (Clipper S) was developed for soil and foliar treatments with a suspension concentrate containing 250 g PAC / l. Soil applications may be as direct soil injection, root injection, basal drenching of plants or, band spraying.

Soil injection uses pressurised equipment to place PAC directly into the root zone around the base of a plant. The alternative is to directly drill into the root system itself but this practice has been largely discontinued as the possibility for introduction of pathogens is also greatly increased.

Basal drenching is perhaps one of the more inefficient methods of soil application in field situations. A trench is scraped around the base of the tree and the compound is poured into this trench. The inefficiency arises from the fact that unless the plant is a known surface feeder (e.g. *Camellia, Rhododendron*), there is unlikely to be any actively absorbing roots in this region of the soil. The technique is best restricted to field situations requiring treatment of only a few trees. Effectiveness of this method is even lower than for soil injection ; retardation of growth in water oak (*Quercus nigra*) after 1 - 1.5 y was 32 % compared to 50 % for the same application rate of PAC when soil injected. In pot trials using small soil volumes, the technique is referred to as soil drenching and this latter method of application has been successful with *Chamelaucium uncinatum* Schauer. (Dawson and King 1993, Stewart 1993), purple nutsedge (*Cyperus rotundus* L.) (Kawabata and de Frank 1993) and, a range of fruit and vegetables (Swietlik and Miller 1985, Reynolds and Wardle 1990, Huang *et al.* 1989).

Band spraying is basically a high throughput extension of basal drenching in which multiple trees have their trunks sprayed with the solution which is allowed to run-off the bark to the soil region. Again, a relatively inefficient method of application with potential for enormous wastage of material along with local contamination.

Soil itself has a large storage and buffer capacity. Many chemicals applied to the soil may be held in reserve for an extended period of time. This may be a useful characteristic if the applicator requires sustained growth retardation over a long period of time. However, in most cases, this persistence is not advantageous and can result in carryover of retardation effects when they are no longer desirable. The persistence of PAC in the soil has already been discussed (Chapter 2) suffice it to say the range of times attributed to PAC persistence vary considerably. Another advantage of most soil application methods (root injection excluded) is that it does not physically damage the plant being treated utilising passive uptake of the material by the plant itself.

The disadvantages of applying chemicals via the soil pathway include; a) production of insoluble precipitates or irreversible fixation or to soil particles or, conversely, b) leaching of applied chemicals from irrigation or, c) competitive uptake and/or metabolism by soil microbes can reduce chemical activity available for plant uptake (Neumann 1988).

In terms of insolubility, PAC has a low solubility in water (35 ppm), and its introduction into all but the driest soils would cause it to crystallise out of solution. PAC itself has been shown to bind to soil particles and has low mobility in most soils (Helling Class 1-2) (Anonymous 1982). Placement of the compound within close physical proximity to the roots of the tree can partially overcome this problem. The binding of PAC in soil is related to the amount of protonated (acidic) organic matter in the soil, binding coefficient may vary from 1.5 on a coarse sandy soil to more than 20 on some high organic matter soils (Anonymous 1982).

The possibility of leaching, in the case of PAC, is unlikely considering its tenacious binding to soil particles and inherently low mobility.

Competitive uptake by microbes is a potential problem. Only very recently have bacteria been isolated which apparently degrade PAC (eight belonging to the genus *Pseudomonas* and one to the genus *Alcaligenes*) (Jackson *et al.* 1996). These bacteria had been enriched from agricultural soils in Tasmania, Australia and are evidently not confined to such situations. This evidence points to the potential for degradation of PAC in the soil.

The entire effectiveness of soil applications of PAC is also dependent on other factors including; volume of soil being treated, distribution and activity of the plant's roots and the ability of the roots to physically take up large molecules such as PAC.

Work with PAC soil drenches is divided between pot trials and field trials. In the case of pot trials, the volume of soil requiring saturation with PAC is a finite quantity, and more precision can be applied to the application of the compound under such situations. Field trials require treatment of large volumes of soil and, by this fact, require greater volumes of compound to be effective. In such cases there is the risk of soil contamination.

The root systems of the plant must also be able to assimilate the compound. While it is beyond the scope of this work to detail root anatomy and physiology, the physical distribution of the root system must be accounted for in a treatment situation. The ratio of young (absorptive) to old (non-absorptive) roots will be critical in the process of initially targeting the compound for greatest effectiveness.

Finally, there is evidence that large molecules such as proteins or polysaccharides do not penetrate into the plant roots (Neumann 1992). Whether this restriction is also placed on PAC is unclear. All we know is that some of the compound must be absorbed by the root systems having passed through a number of environmental filters (i.e. precipitation, degradation, physical translocation).

To illustrate the relative inefficiency of soil uptake, a study was conducted in which water oak (*Quercus nigra*) was treated with either soil injected Clipper S or trunk injected Clipper T. The percentage retardation of growth 1 - 1.5 y after treatment was 74 % for trunk injection but only 60 % for soil injection (Anonymous 1982).

The final component of the soil drench efficiency equation rests with the plant species being treated. The variability of response to PAC is well documented ranging from non-responsive (*Fraxinus*, *Cupressus*) to short term (*Jacaranda*, *Salix*) and long term responsive species

(*Liquidambar*, *Acer*). This is perhaps the most puzzling aspect of the equation because we have no suitable evidence to demonstrate exactly *why* this variability occurs and, this is an area of PAC research that requires further attention.

4.2.2 Foliar application

The alternative application of Clipper S is as a foliar spray. To its advantage, foliar sprays like soil applications, do not physically damage the tree thus reducing the risk of introducing pathogens. In addition, uptake can occur in a matter of hours rather than days, weeks or months in the case of soil applications. The compound is also applied much nearer the sites of activity (i.e. the apical and lateral meristems). The final advantage is that the compound is more uniformly distributed over the plant rather than introduced as isolated patches as with soil application. With this many advantages, foliar application of PAC would seem the ideal method of application and has, indeed, been adopted by many researchers as the method of choice for application of PAC.

Foliar sprays have been successfully utilised to retard growth on woody shrub species (Keever *et al.* 1990), herbaceous perennials (Banko and Stefani 1988), (Keever and Cox 1989) and trees (Horowitz 1992). However, there are also disadvantages to this method of application.

First of these is the actual permeability of leaves to foliar sprays. Surrounding the leaf is a protective cuticle which is a complex mixture of pectins, cutin, fatty acids and waxy alcohols (Kolattukudy 1970). The water repellency characteristics of this layer is high and requires the addition of surfactants to increase initial absorption to the leaf surfaces. In addition, the intrinsic permeability of cuticles can vary with leaf age, environment and plant variety which makes difficult the task of determining spray rates and amount of spray solute uptake. Photo-oxidative effects and surface catalysed decomposition of compounds are also cited as being problems but in the case of PAC neither is a serious issue. If the applied compound can overcome the cuticle barrier it is rapidly absorbed and translocated within the living plant.

As the solution containing PAC evaporates the rate of absorption drops markedly. Also, rates of diffusive penetration into the leaf decreases as the molecular size of the penetrating solution increases (Cutler *et al.* 1982). In the case of PAC one study has shown that its cuticular sorption and penetration characteristics were rapid (24 h at 25 °C) in pear (*Pyrus communis* L.), vanilla (*Vanilla*), tomato (*Lycopersicon esculentum* Miller) and capsicum (*Capsicum annuum* L.) (Chamel *et al.* 1991). This would suggest that penetration and sorption is not an important issue when applying PAC.

The final disadvantage attached to foliar sprays is the possibility for spray drift contaminating non-target plants or surfaces. One recent advance which may minimise this risk involves the electrostatic 'charging' of sprayed compounds to enhance their attraction, and adherence to, the leaf surface.

The majority of research into PAC effects relies on application of PAC as a foliar spray. However, in a large scale experimental system using many small plants or a field situation involving fewer large plants foliar application is not an attractive option.

4.2.3 Trunk injection

4.2.3.1 Introduction

Trunk injection techniques have been used extensively to incorporate not only growth retardants, but also insecticides, fungicides, herbicides and nutrients into plant systems (Himelick 1972). A comprehensive review on this subject was conducted by Roach (1939). Naturally enough, the target plant must have the size and mechanical strength to support trunk injection and hereafter reference to this application method is restricted to woody shrubs and trees.

The first documented use of trunk injection dates to the time of Leonardo da Vinci in 1158. da Vinci injected his apple trees with an arsenical fluid to prevent theft of the fruit (Roach 1939). The success or otherwise of this initial experiment have not been recorded for posterity.

There are a few approaches by which material can be introduced into the trunk of a tree. The first is through passive diffusion of material from gravity feeding of solutions. An example of what can be achieved using this technique may be found in work by Duncan and Himelick (1990). The second process is active, through the pressurised injection of solutions. This latter process will be dealt with in some detail as it is the basis of the field trial accompanying this chapter.

The variety of trunk injection equipment systems available at present vary only slightly in their usage. All trunk injection requires penetration of the outer layers of the tree by means of an augur or drill. A hole is made through the external layers to the zone of active xylem immediately beneath the vascular cambium. The number of drill holes required is proportional to the circumference of the tree to be injected. The angle of the drill hole is at approximately 45° to the trunk surface. Shallower than this and the solution is injected into non-conductive tissue. Too deep and the functional xylem may be bypassed (Figure 4.1). Although the technique

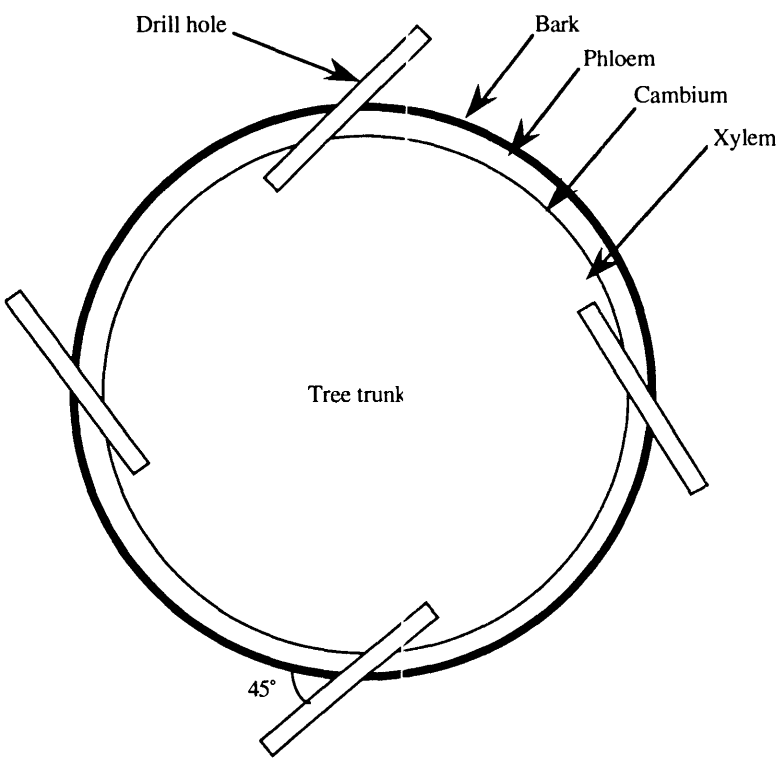


Figure 4.1 Schematic representation of drill hole insertion for trunk injection of PAC

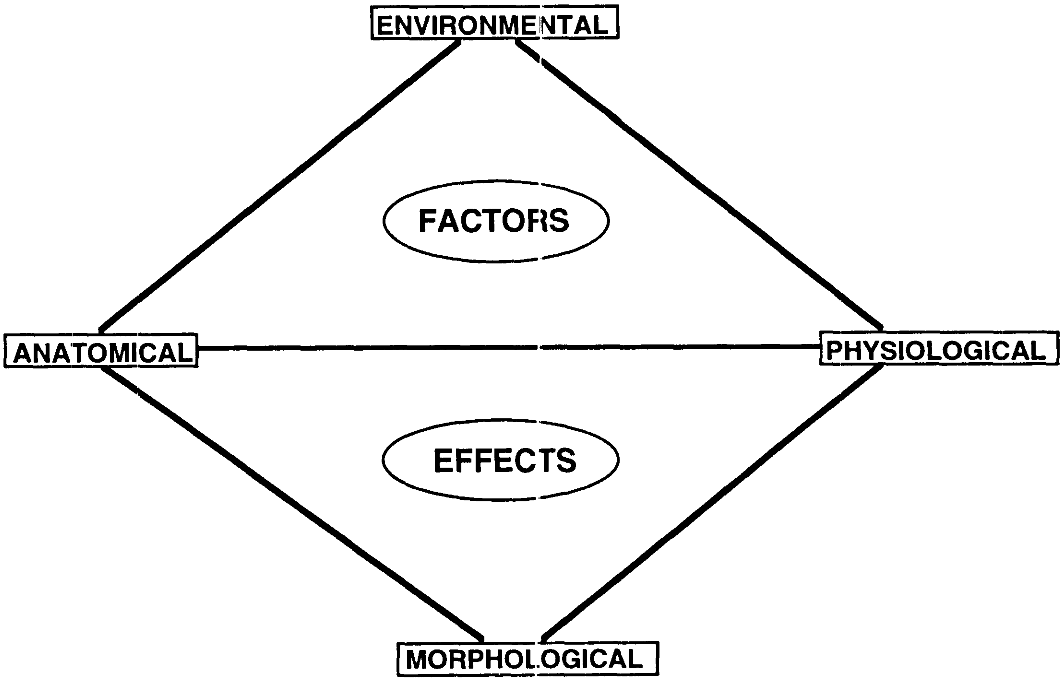


Figure 4.2 Pre-injection / Injection conditions (factors) and post-injection conditions (effects) influencing effectiveness of trunk injection of PAC. See text for a fuller explanation of parts. Both anatomical and physiological factors affect response pre- and post-injection.

would appear imprecise, very effective results have been obtained using this methodology.

Before, during and after trunk injection of PAC there are a number of conditions which ultimately influence the compound's effectiveness (Figure 4.2). Before injection and at injection time, these conditions can be grouped into three main areas; anatomical, physiological and, environmental. After injection (post-injection) the conditions influencing PAC effects can also be grouped into three areas; anatomical, physiological and, morphological. Both anatomical and physiological conditions are influential before, during and after trunk injection. Environmental factors relate to the pre-injection condition of the tree while morphological factors affect only the post-injection condition of the tree. The issues surrounding each of these conditions shall be discussed in more detail.

4.2.3.2 Pre-injection & Injection considerations

4.2.3.2.1 Anatomical

Prior to injection there are factors involving the wood anatomy of the tree which can have a significant effect on the movement and distribution of injected compounds. The main factors relate to wood density, wood porosity and grain.

Density of wood. There is an inverse relationship between wood density and injection rate. For example, basswood (*Tilia americana* L.) and black cherry (*Prunus serotina* Erhr.), in which injection rates were high, have much lower specific gravity than black locust (*Robinia pseudoacacia* L.) and sugar maple (*Acer saccharum* L.), in which injection rates were low (Sinclair and Larsen 1981).

Ring vs. diffuse porous wood. (Huber 1935) has shown that the velocity of sap ascent in ring-porous trees is nearly ten times greater than for diffuse-porous trees. Conduction of water took place only in the outer ring of ring-porous trees but in a much larger area than in diffuse-porous trees. Ring porous woods have wider and longer vessels, more heartwood and conduct water much faster in only a narrow zone of the outermost growth ring. Diffuse porous woods have narrow and short vessels, less heartwood and conduct water more slowly in a larger transverse sectional area (Huber 1935). One of these observations was corroborated by (Greenidge 1952) who found that vessels in ring-porous species are longer than those in diffuse-porous species. The implications of these findings for trunk injection are that the distribution of vessels in a tree should be considered prior to injection so that optimal distribution of PAC can be obtained.

Straight vs. spiral grain wood. Wood grain falls into a number of categories (Rudinsky and Vite 1959) but for the purpose of this discussion only straight grain and spiral grain are important. Straight grained trees have continuous vasculature from the root on one side of the tree to twigs on the same side (Zimmermann and Brown 1971). Spiral grained trees have vasculature which does not run longitudinally, but twists resulting in a cross-grained effect. Trees with straight grained wood will require a greater number of injection points to enhance distribution of injected materials. Spiral grained woods will require fewer injection sites as injected material is more widely distributed about the tree.

Xylem vessel characteristics. Consideration should also be given to the influence of xylem vessel width, length and frequency within the wood to be injected. There is a correlation between wider or longer or more frequent vessels and increased rates of uptake. Another documented correlation is between uptake and wood density. The wood porosity index, developed by (Sinclair and Larsen 1981) is calculated as relative frequency of xylem vessels in cross-section / specific gravity. This index was significantly correlated with average injection rate ($r = 0.93$, $p = 0.01$) in 13 angiosperms tested.

Non-porous woods. Trees with more 'primitive' anatomical arrangements (i.e. conifers) are a special case in themselves and vary greatly in their response to injected plant growth retardants. The anatomical arrangements of xylem elements differ, with conifers consisting entirely of tracheids with an anatomy referred to as non-porous. Vite and Rudinsky (1959) injected the dye acid fuschin into 23 species of conifer and identified five different patterns of translocation (left-turning spiral ascent, right-turning spiral ascent, interlocked, sectorial straight, sectorial winding) in felled sections. This highlights the variability in conifer anatomy and potential problems with translocation of injected materials. Wheeler (1987) reported soil drenches of PAC significantly reduced growth of container-grown Douglas fir (*Pseudotsuga menziesii* Mirb. Franco) and loblolly pine (*Pinus taeda* L.) but did not affect growth when injected into 3 - 9 year old trees. Sinclair and Larsen (1981) injected a range of species with water at 0.7 kg / cm^2 (10 psi). *Pinus strobus* (eastern white pine) yielded near zero flow rates per injection. Finally, transpiration in conifers is lower than in the majority of angiosperms with slower peak velocities recorded with the compensation method (Zimmermann 1983). All of this evidence confirms that conifers generally are not good candidates for trunk injection.

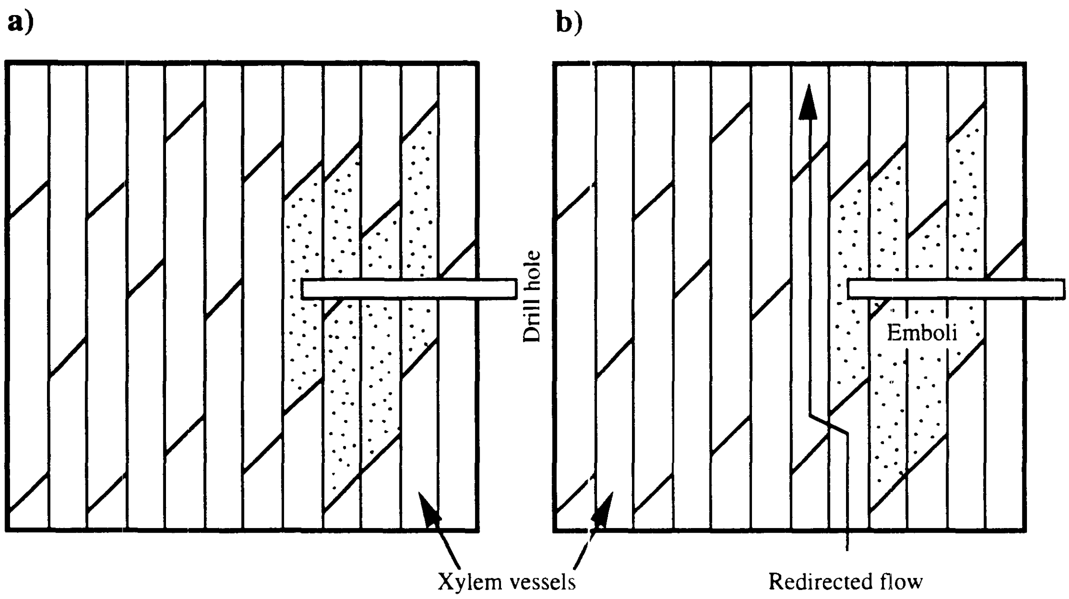


Figure 4.3 Xylem vessels **a)** before and **b)** after disruption from drilling. Emboli (stippled areas) form in the vessels surrounding the drill hole but are restricted by pit membranes.

4.2.3.2.2 Physiological

Within the plant, the xylem contains a continuous column of water from the roots to the leaves. The vessels and tracheids making up the system are dead at maturity and act like a series of pipes. This arrangement reduces the complication of movement through membranes which can impede conduction considerably. The only disruption to this process is the result of entry of external atmosphere to the system or creation of sufficient negative tension to allow gases in solution to escape into the vapour phase (Figure 4.3). This process is known as cavitation, and commences in most plants at water potentials around -1 to -2 MPa. Gas bubbles (emboli) resulting from cavitation are unable to withstand tensile forces and expand once created. This expansion is checked by the pit membranes and occluded vessel members are isolated. Water conduction continues around the isolated vessel through parallel, interconnected vessel members.

Thus, the initial physiological reaction of a plant to xylem damage is the dissipation of negative tension in a localised area followed by cavitation, and the production of emboli with the result that conduction of materials at these points is completely impeded. The emboli can only be reabsorbed naturally when the xylem tension is lower, which may occur with the assistance of root pressure.

The immediate problem with trunk injection is to overcome the attendant resistance within the vessels and tracheids. This is achieved through the use of pressurised injection equipment. Pressurisation is useful in forcing air emboli caused by disrupting the xylem, back into solution allowing for a continuous interface between sap and compound. However, it should be noted that the application of positive pressure is only a temporary state and once released may allow these bubbles to re-form. There are many units available for pressurised injection of compounds. They consist of a gas reservoir, multiple injection heads and a method of injecting measured quantities of solution. One unit is the Philips Multi-Head Injector (N.J. Philips, Gosford) which was designed specifically for use in conjunction with Clipper (Plate 4.1).

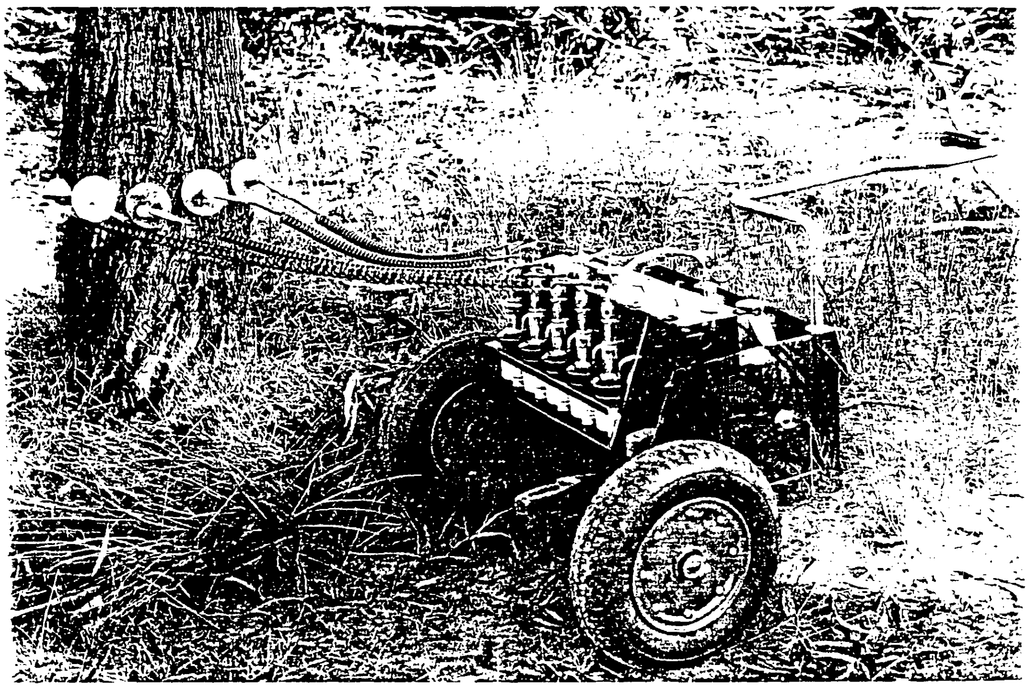


Plate 4.1 The Philips Multi-Head Trunk Injection machine produced by N.J. Philips, Gosford and used in these field trials

The actual pressure required to force solutions into trees is also dependent on tree health, atmospheric temperature and humidity, with the consequence that actively transpiring trees will assimilate the injected solution more rapidly. Unhealthy trees should never be injected as it only compounds stress already placed upon the organism. Likewise, there is a recommendation to never inject a tree when temperatures exceed 35 °C.

A range of pressures have been generated for trunk injection experiments from as low as 0.61 - 0.81 kg / cm^2 (60 - 80 kPa) (Navarro *et al.* 1992) to 7.0 - 14.0 kg / cm^2 (689 - 1378 kPa) (Riel 1979) up to as high as 25 kg / cm^2 (2460 kPa) (Sterrett and Creager 1977). Dementiev (1914) found that most trees could safely cope with pressures of around 6 atmospheres (607.8 kPa). Where pressure exceeds the mechanical strength of the wood a phenomenon known as bark blowout can occur in which the outer layers of the trunk are literally sheared apart by the pressure generated. The currently recognised 'safe' level is considered to be around 5.5 kg / cm^2 (550 kPa).

Following injection of material the drill hole is routinely sealed with a mastic compound to eliminate continued exposure to the surrounding atmosphere and thus, potential pathogens. This, combined with the presence of the alcohol carrier solution of PAC mitigates the possibility of establishment of pathogens.

4.2.3.2.3 Environmental

Apart from the issues already raised there is also the question of what time of year the application takes place. For deciduous tree species, there are really only two seasons available during which trunk injection can be conducted; spring and autumn. During winter the plant is in a leafless state and unable to accept material while in summer, in many parts of Australia, air and/or soil temperatures are likely to exceed 35 °C, the maximum considered 'safe' for trunk injection. Evergreen species can additionally be injected on warmer days during winter. Cox (1990) found that injection of *Lophostemon confertus* ((R. Br.) Peter G. Wilson & Waterhouse) in May (mid autumn) or July (early winter) was not as effective as in March (late summer) or October (early spring).

Finally, in considering the timing of the application, we must also consider recent tree history and the broader picture of its growth and metabolism on a seasonal basis.

Tree history may include information on recent weather conditions, fertiliser regime (if any), levels of disease or infestation of individual specimens and, pruning regime. Weather conditions provide useful information about the degree of stress the tree may be experiencing. For example, if local drought were prevalent it would not be prudent to inject a tree; low soil moisture levels generally translate into reduced transpiration and therefore, reduced uptake and distribution of trunk injected material. Fertilisers will alter the nutrient balance of the tree to the extent of promoting vegetative or reproductive growth. Excessive vegetative growth at the time of injection might be advantageous with translocation of material to the active sites of cell division. Alternatively, excessive leafiness may increase transpirational losses from the plant thus imposing stress. Diseases and pests reduce the vigour of a tree and place it under stress. As with high temperature/low moisture conditions, PAC should never be applied if this is the case. Finally, pruning regime relates to the frequency and severity of pruning experienced by the tree prior to injection. It is currently recommended that trees be pruned four to six weeks prior to trunk injection of PAC for maximum growth retardation.

4.2.3.3 Post-injection considerations

4.2.3.3.1 Anatomical

One post-injection reaction of the tree involves a process known as compartmentalisation, in which diseased or damaged trees naturally form boundaries separating decayed or disrupted wood from sound wood. An elegant model was developed by Shigo (1984) called compartmentalisation of decay in trees or, CODIT. CODIT defines four individual stages of wall formation (barrier zones) which follow production of reaction zones (incorporating antimicrobial substances) around the affected area. The practical significance of compartmentalisation is to give the tree the opportunity to survive for long periods under stress of periodic injury or infection (Shigo 1984).

The degree and severity of the wounding will depend on the chemical being introduced, method of introduction and timing of introduction. In respect of the latter statement, Perry and Hickman (1987) found that wound closure in *Eucalyptus* was more rapid in spring than in late autumn or winter. Neely (1970) found that while winter and spring wounds healed more slowly than summer or autumn wounds the propensity for dieback was highest in the latter seasons. Neely (1987) summarised reports on this subject by saying that i) wound closure is directly related to tree vigour, ii) large wounds close more slowly than small wounds (wounds < 12 mm diameter are likely to close in 1 year) and that, iii) annually inflicted wounds < 25 mm in diameter are not likely to slow tree growth. Thus trunk injection elicits the same response as pathogenic infection and the tree responds appropriately.

4.2.3.3.2 Physiological

Movement and distribution of PAC. PAC injected into the trunk of a tree has been monitored by several researchers (Quinlan and Richardson 1986, Wang *et al.* 1986b, Intrieri *et al.* 1987, Early and Martin 1988). The monitoring of PAC at point sources is adequate but the actual pathway taken within the plant is unclear.

Current theory suggests that at the point of injection, PAC crystallises out of solution and adheres to the walls of the xylem elements (vessels / tracheids). Initially, only a small amount is transported to the apical meristems. The crystallised PAC is then slowly eluted to the meristem by the transpirational flow in the xylem (Anonymous 1982). Owing to its low solubility this release can occur over many months or years. Use of the term adhesion in respect of the deposition of PAC may not be strictly correct. It is known that the PAC molecule does not carry a charge and it may be more a case of physical impedance at the sieve plates than adherence that prevents the majority of crystallised material from moving upwards.

Factors affecting this release include i) environmental conditions influencing the plant, ii) growth rate of the plant and, iii) health of the plant. Some of these factors are interrelated and have been previously discussed.

A second method of movement of PAC may involve diffusion. Diffusion is the process by which molecules move from an area of high concentration to one of low concentration. Diffusion is not an issue in mass transport over long distances but can be important at the cellular level. Surrounding vessel members are other vessel members connected by pits. Diffusion of solubilised PAC may occur over these small distances. Slowly spreading outwards from the point of origin (i.e. injection hole) PB may move into several vessel members/ tracheids or parenchymatous tissues.

The final phase in the transport of PAC occurs from shoots to leaves and the apical meristem. PAC is only effective once the reserve of active gibberellin (GA_3) have been depleted. Thus, PAC does not prevent GA from fulfilling its biological function, but inhibits biosynthesis of more of the compound.

Sequestering of PAC. The relative efficiency of conduction of injected PAC is questionable but theorising about the movement of PAC in plants is made simpler by the fact that there are no living cells or membranes to impinge on the conduction of the compound in the xylem.

Finally, at the point of introduction PAC may also become sequestered. This process occurs with the normal growth of the plant as it expands laterally by production of secondary tissues by the vascular cambium. By this process, conductive tissues are renewed, and the plant develops sufficient mechanical rigidity to support further vertical growth. Undissolved PAC adhering on the xylem walls may be lost to the system once this tissue is made non-functional through production of new conductive tissue. Often the vessels or tracheids will naturally become non-functional with the deposition of cellular products or ingrowth of tyloses which render them inefficient water conductors. Sequestering is more likely to occur if the plant is injected prior to a period of active growth.

4.2.3.3.3. Morphological

The range of morphological effects PAC can have on plants has been discussed in-depth in a previous chapter (Chapter 2).

4.2.3.4 Disadvantages of trunk injection

To maintain equity and conclude this discussion, it is worth noting that there are disadvantages to trunk injection of compounds. Firstly, heavy equipment is required to generate the pressure necessary for the injection process. However, the equipment is also easily controlled and maintained. The remaining disadvantages have been discussed earlier in this section but relate mostly to efficiency of uptake and distribution relating to site of application, trees species or variety and, stage of growth.

4.3 Street Tree Trials with PAC

Electricity distributors are required by law to keep trees out of powerlines. Overhead Line Construction Regulation 38 (1962) states;

"Where an aerial conductor of an overhead line or overhead service line is in contact or is likely to come into contact with any tree, steps shall be taken by the electricity supply authority to have such tree trimmed to prevent contact with the aerial conductor..."

Expenditure by electricity distributors on pruning and maintenance of trees under powerlines is high. For example, since its inception in 1977, SEQEB (South East Queensland Electricity Board) has spent $ 3 million annually on controlling tree growth near overhead powerlines. On a smaller scale, New England Electricity (now incorporated into NorthPower) has an annual expenditure of $ 300,000 on tree trimming. At the other extreme, the Florida Power and Light Company, serving over 3.1 million customers had an annual expenditure in 1990 of over US$ 17 million (US$27.50 per tree) in line control programs (Tamsberg 1990). There is clear evidence that manual tree pruning and maintenance is very expensive in terms of direct financial expenditure.

Apart from financial consideration is the unattractive state of severely pruned trees. Such pruning is unsightly (Plate 4.2), the severity of which is often considered by the public to be wholly unnecessary. Even though pruning trees under powerlines is a necessary requirement by law, negative public reaction is often unavoidable.

Despite public resistance to the practice, trees under powerlines must be pruned. If they are allowed to grow into lines the branches pose a hazard to electricity supplies by shorting wires or, dragging lines down in windy or stormy weather (Plate 4.3).

Plate 4.2 An example of a poorly pruned specimen of *Eucalyptus nicholii* under powerlines.

Plate 4.3 Detail of canopy of *Eucalyptus nicholii* showing branches reaching into powerlines.

An alternative to manual pruning that has been trialed successfully is through trunk injection of plant growth retardants, including PAC. Unfortunately, the majority of experiments by other electrical distributors are anecdotal and have not been scientifically documented. Using the Florida Power and Light Company example, implementation of a tree injection program successfully retarded growth of trees under powerlines. Growth retardation ranged from 62.5 % to 90 % over control (uninjected) trees (Tamsberg 1990).

In Australia, growth retardants have been trialled by not only electricity distributors (e.g. SEQEB, Wide Bay-Burnett Electricity, ETSA (Electricity Trust of South Australia), Prospect Electricity) but also city and local councils (e.g. Melbourne City Council, Tamworth City Council). Again, the inherent problem is that the trial data has not been externally evaluated and published making it difficult to access relevant material for comparison. Recommendations for injection of some amenity trees are listed below in Table 4.1.

Some documented and published trials were conducted by Deans (1989) in South Australia under the auspices of ETSA. Although these trials were conducted on 20 species of tree the only results presented refer to *Eucalyptus oleosa* F. Muell. ex Miq.and *E. socialis* F. Muell. ex Miq. in which growth was retarded by an average of 70 % compared to controls. English oak (*Quercus robur* L.) was retarded by 60 - 70 % compared to controls.

Given the lack of scientifically documented evidence available it was appropriate to conduct a series of local street tree injection trials to evaluate the performance of PAC. These trials were conducted under the auspices of the then New England Electricity (now NorthPower) and with assistance from Armidale City Council. The trials were to determine whether PAC is a suitable alternative option to manual pruning in some of the more problematic street trees in the City of Armidale.

4.4 Materials and Methods

4.4.1 Species selection

Three tree species were identified as causing potential problems under powerlines in the City of Armidale (30 ° 32' S, 151 ° 38' E), New South Wales, Australia. These species were ; *Eucalyptus nicholii* Maiden & Blakely (Family Myrtaceae) (eulines.tif - Figure 4.5), *Liquidambar styraciflua* L. (Family Hamamelidaceae) (lslines.tif - Figure 4.6) and *Pistachia chinensis* Bunge (Family Anacardiaceae) (pistachia.tif - Figure 4.7). Over 50 % of street trees in the City of Armidale are *Pistachia*, while *Liquidambar* (7.7 %) and *Eucalyptus* (3.5 %) are less common. The tallest growing species are *Liquidambar* (40 m), *Eucalyptus* (18 m) and *Pistachia* (11 m). The clearance of overhead powerlines is 4.5 m. Site selection of these

Table 4.1 Recommended dosage rates of PAC in a range of amenity tree species . Note that injector spacing is specified but is generally 10 or 15 cm. This will depend on actual circumference of the trunk (data from various sources).

Scientific name	Common name	Volume of Clipper (ml / hole)	Injector spacing (cm)
Casuarina equisitifolia	Australian Pine	35-75	20
Euc. camaldulensis	River Red Gum	50	15
E. globulus	Tasmanian Blue Gum	100	15
E. leucoxylon	Sth. Aust. Blue Gum	60	15
E. odorata	Peppermint Gum	50	15
Grevillea robusta	Silky Oak	50	10
Lophostemon confertus	Queensland Brush Box	80	10
Melia azederach	White Cedar	75	10
Populus nigra	Black Poplar	35-75	10
Salix babylonica	Weeping Willow	35-75	15
Ulmus parvifolia	Chinese Elm	35-75	20
Ulmus procera	English Elm	25	10

Euc. = *Eucalyptus*

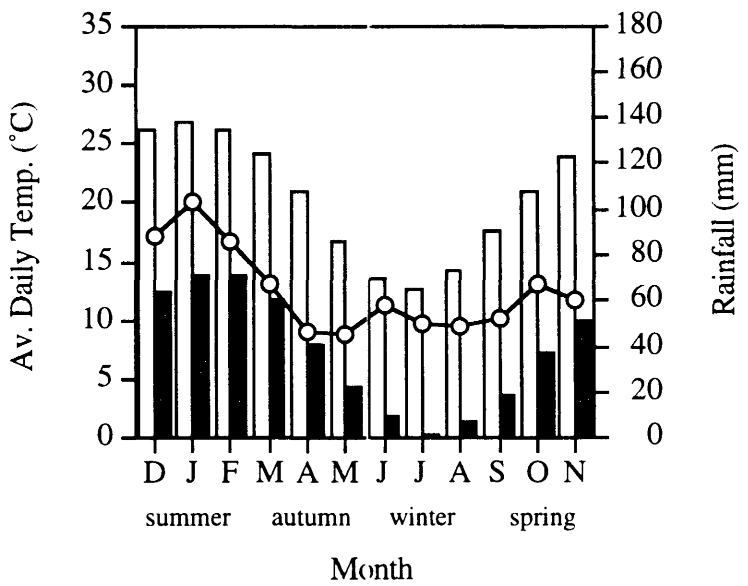


Figure 4.4 Average weather conditions in Armidale (30°32'S, 151°38'E, 980 m asl). Temperature indicated by white (av. daily max.) and grey (av. daily min.) bars. Rainfall (740 mm) is summer dominant.

Plate 4.4 *Eucalyptus nicholii* as a street tree in in the City of Armidale (see text).

Plate 4.5 *Liquidambar styraciflua* as a street tree in the City of Armidale (see text). Note the proximity of the overhead powerlines in relation to the canopy.

Plate 4.6 A mature specimen of *Pistachia chinensis* growing in the grounds of the University of New England, Armidale.

4.4

4.5

4.6

nicholii and *P. chinensis* were slightly higher than for *L. styraciflua* which has been noted to be 'sensitive' to high concentrations of PAC (Table 4.2). The allocation of each concentration within each species was randomly assigned. Coloured metal plates were attached to the trunk to signify the concentration applied. The experimental design is summarised in Figure 4.5.

4.4.4 *Trunk injection of PAC*

Trees were trunk injected during a three week period in October. A Makita® cordless drill with a 6 mm wood bit was used to bore holes into the trunk at 45° to the trunk surface and in a slightly downward direction. This targeted the layer of actively transporting xylem immediately below the phloem and cambium (see Figure 4.1).

Clipper® was injected into tree trunks using a Philips Multi-head Trunk Injection machine (N.J. Philips P/L, Gosford, N.S.W.). The machine operates through the pressurisation and depressurisation of a piston / cylinder head which draws on a reservoir of the solution being injected. The volume of material injected was adjusted to 25 ml / cylinder and the pressure maintained at 5.1 kg / cm^2 (500 kPa). Based on previous trials, one injector point was allocated for every 15 cm of trunk circumference.

The speed with which the tree assimilates the injected material was dependent on a number of factors. These may include light, temperature, humidity, soil moisture availability and tree health. These trials were commenced in spring with trees actively transpiring. Assimilation of material ranged from 5 s to 30 s while the average was 10 s.

Drill holes were sealed with a brown mastic compound to minimise introduction of pathogens through the wound site.

4.4.5 *Measurements of the effect of PAC*

Prior to injection with Clipper®, numbered plastic tags were attached to 20 shoots on each tree. Three measurements were recorded: i) leaf number, ii) internode length (containing two subsets; above and below) and, iii) number of leaves present on each shoot.

Leaf number was recorded by counting the number of live leaves present on the tagged shoot. Leaf number was recorded to examine if Clipper® was affecting their rate of production. Leaf number was recorded as a whole figure.

Internode lengths were recorded by selecting the second oldest node from the tagged shoot. The length above (upper internode) and below (lower internode) this node were measured.

Lengths were recorded in mm. This measurement would examine whether Clipper® targeted cell expansion/elongation in this region of the shoot.

Shoot length was measured from the base of the current season's growth to the apex of the shoot. Seasonal growth is easily identified by expansion and compression of the stem. The bark is also of a lighter colour in new growth. Shoot length was measured in cm and would demonstrate how effectively regrowth was being reduced between concentrations.

Data was collected at 0, 3, 12, 15 and 18 months and analysed using a standard Analysis of Variance (AOV) using the program Statview® (Abacus Concepts, Inc. 1992) for the Macintosh. These times of measurement were chosen to coincide with peak growth periods of all species.

4.5 Results

There was no significant difference within each species between concentrations for internode length or leaf numbers. Leaf numbers could be directly attributed to the extension or suppression of shoot length. The results for these measurements are not presented here.

Growth retardation over the entire experimental period for each species is shown in Figure 4.6 (*Eucalyptus*), Figure 4.7 (*Liquidambar*) and Figure 4.8 (*Pistachia*). Significant differences within species between treatments are listed in Table 4.3.

Of the three species tested, *Liquidambar* showed the greatest response to PAC during the 18 month trial (Figure 4.7). Differences in shoot length can be seen 3 months after injection with PAC with the control treatment and the 50 g / l and 25 g / l treatments being significantly different. With increasing concentration of injected PAC there is increasing reduction of shoot length.

After 18 months, it was shown in *Pistachia* that 50 g / l PAC and 75 g/ l PAC produced a similar effect in reducing shoot length (Figure 4.8). Similarly, 12.5 g / l PAC and 25 g / l PAC produced a similar response while for all concentrations the reduction in shoot length was significantly different from the control.

Eucalyptus was the least responsive test species. No significantly different response in shoot length was elicited by PAC after 3 months or 12 months following injection (Figure 4.6). Some differences appear in successive measurements but these are not as dramatic as the responses shown in *Liquidambar* or *Pistachia*. Treatment with 75 g / l PAC produced the highest reduction of shoot growth while 0 g / l PAC, 12.5 g / l PAC and 25 g / l PAC were not significantly different from each other.

Table 4.2 Concentrations of PAC used for each species. *Liquidambar* concentrations were lower due to its reputed sensitivity to PAC.

Species	PAC Concentration (g / l)				
	Control	1	2	3	4
Eucalyptus	0	12.5	25	50	75
Liquidambar	0	5	12.5	25	50
Pistachia	0	12.5	25	50	75

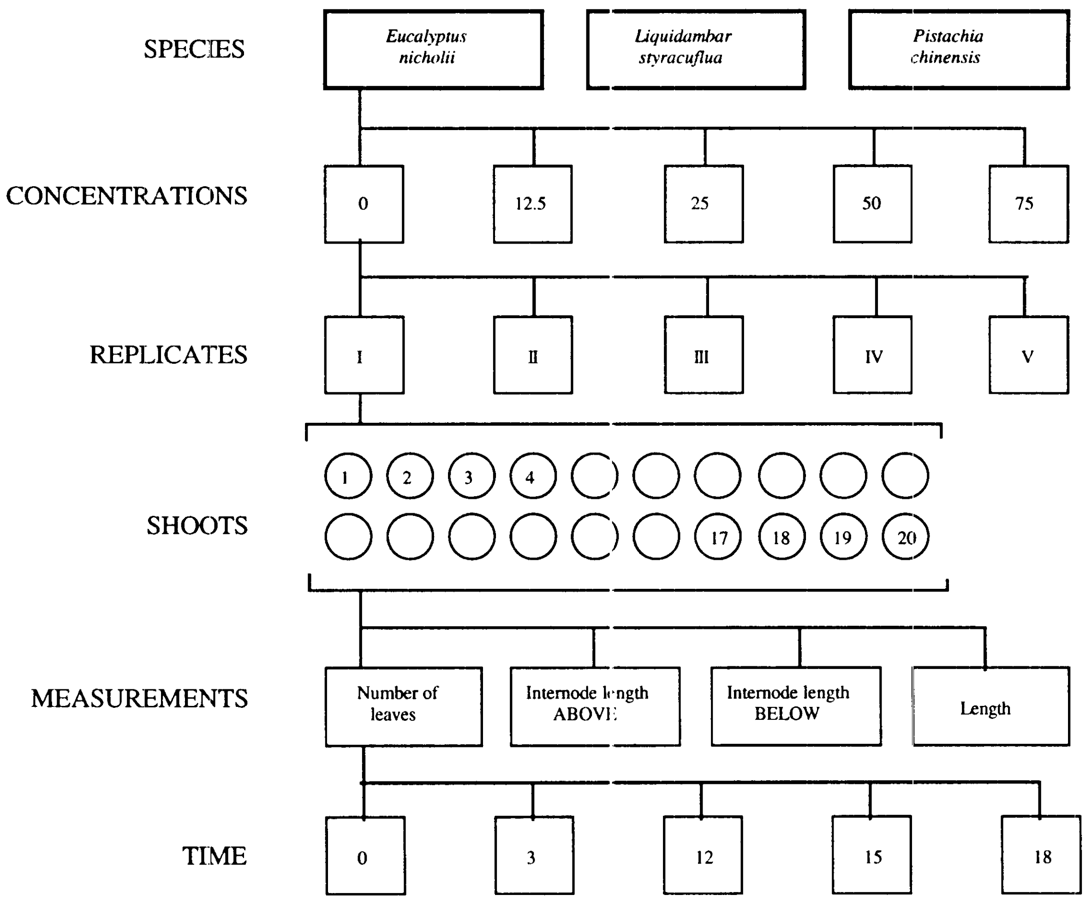


Figure 4.5 Summary of experimental design for street tree trials with PAC

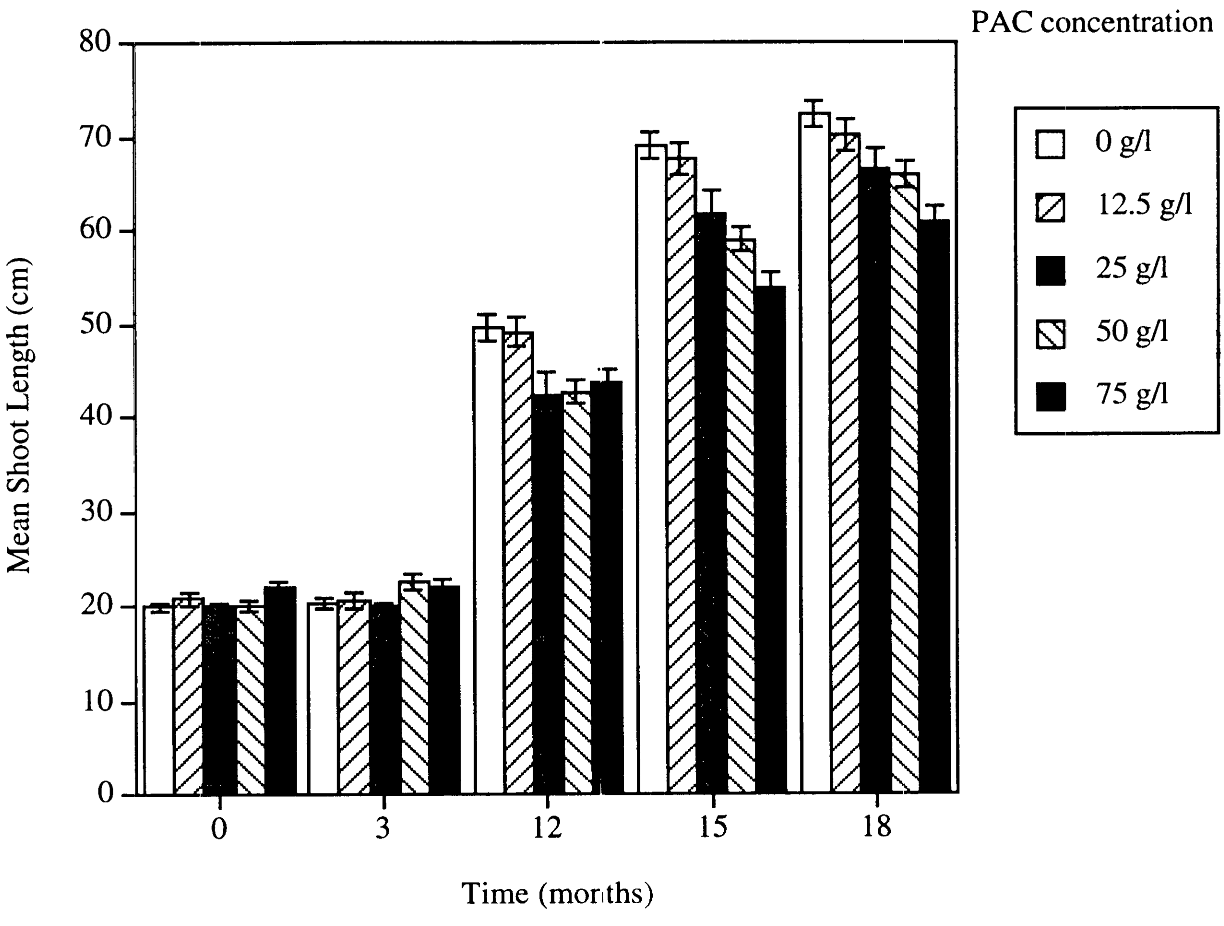


Figure 4.6 Growth retardation of mean shoot length over 18 months resulting from trunk injection of PAC in *Eucalyptus nicholii*. Bars indicate standard error. Significant differences between treatments are listed in Table 4.3.

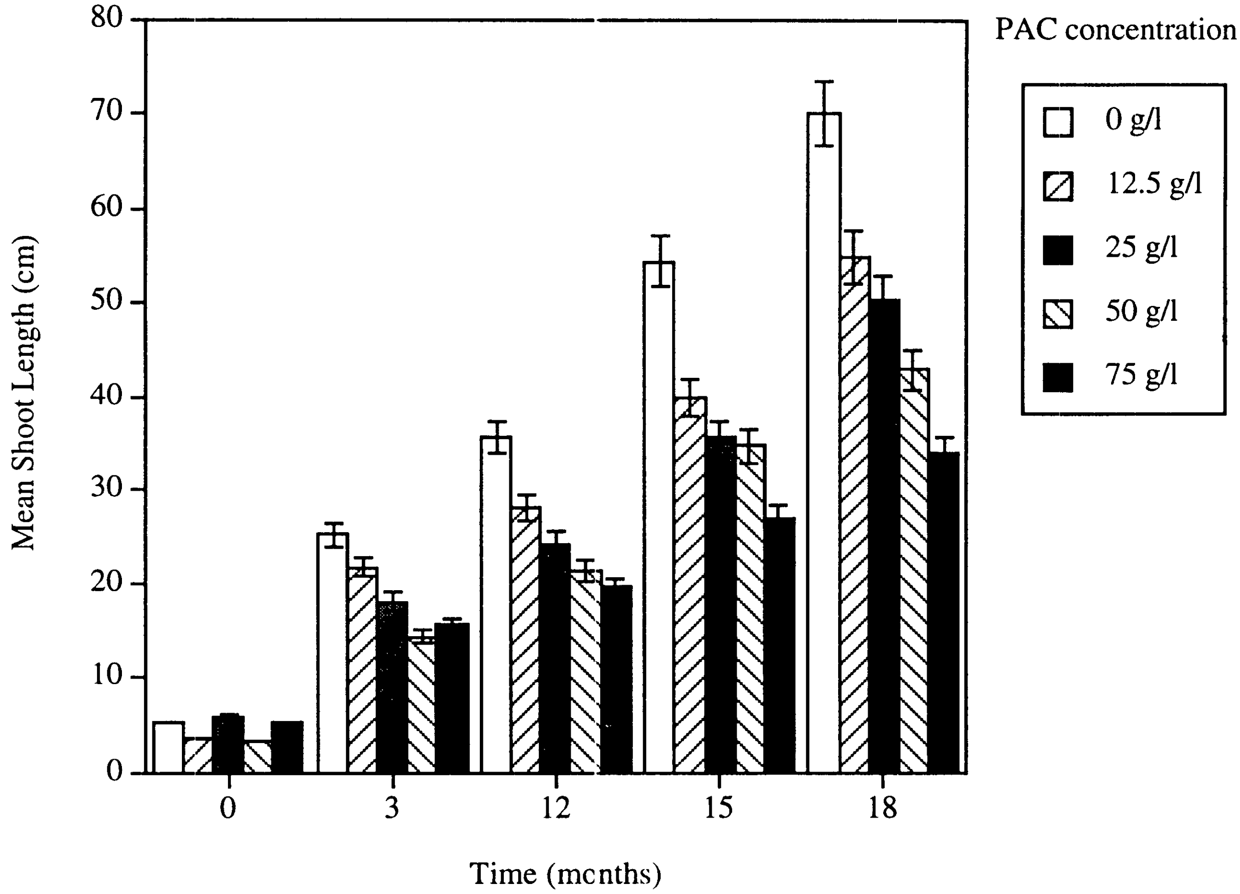


Figure 4.7 Growth retardation of mean shoot length resulting from trunk injection of PAC in *Liquidambar styraciflua*. Bars indicate standard error. Significant differences between treatments are listed in Table 4.3.

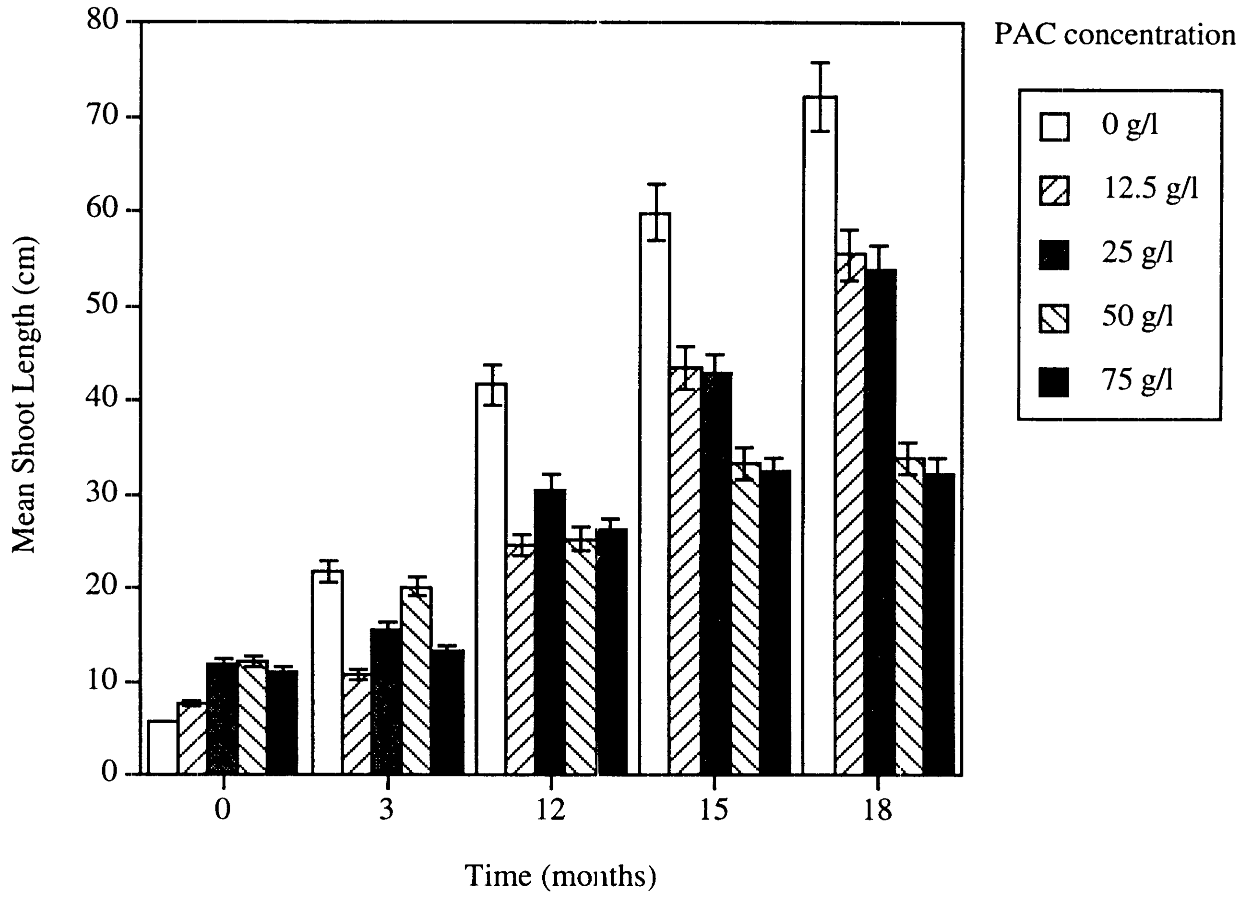


Figure 4.8 Growth retardation of mean shoot length resulting from trunk injection of PAC in *Pistachia chinensis*. Bars indicate standard error. Significant differences between treatments are listed in Table 4.3.

Table 4.3 Mean shoot length (cm) in three tree species as affected by injected PAC over 18 months. Means followed by the same letter are not significantly different (P≤0.05) as determined using Scheffe's (1953) S method.

Species	*Time (months)*	*PAC concentration (mg/l)*				
		0	*5*	*12.5*	*25*	*50*
Liquidambar	*0*	5.27*a*	3.66*a*	5.72*a*	3.18*a*	5.27*a*
	3	25.15*a*	21.76*ab*	18.09*ab*	14.39*b*	15.61*b*
	12	35.74*a*	28.22*b*	24.25*bc*	21.42*bc*	19.65*c*
	15	54.36*a*	39.86*b*	35.59*bc*	34.73*bc*	26.98*d*
	18	70.10*a*	54.87*b*	50.45*b*	43.00*c*	34.02*d*
		0	*12.5*	*25*	*50*	*75*
Pistachia	*0*	5.62*a*	7.62*a*	11.93*a*	12.07*a*	11.09*a*
	3	21.67*a*	10.69*b*	15.45*ab*	20.14*ab*	13.19*ab*
	12	41.64*a*	24.54*b*	30.56*b*	25.22*b*	26.12*b*
	15	59.84*a*	43.40*b*	42.76*bc*	33.16*cd*	32.30*d*
	18	72.23*a*	55.39*b*	53.82*b*	33.87*c*	32.79*c*
Eucalyptus	*0*	19.91*a*	20.83*a*	19.94*a*	20.12*a*	22.14*a*
	3	20.22*a*	20.87*a*	19.99*a*	22.53*a*	22.19*a*
	12	49.66*a*	49.13*a*	42.40*a*	42.72*a*	43.65*a*
	15	69.19*a*	67.65*ab*	61.76*ab*	59.07*b*	53.97*c*
	18	72.56*a*	70.28*ab*	66.43*ab*	65.98*b*	61.00*c*

4.6 Discussion

4.6.1 Physiological response to PAC

Growth retardation. This experiment has shown that PAC reduced shoot length in the three species tested. However, the degree of reduction varied between individual species.

In *Liquidambar* the reduction in shoot length in response to PAC is significant (Table 4.3). After only 3 months there is a significant difference in shoot length between the control and the other treatments (Figure 4.7) (Plate 4.7). At 18 months this difference is significant between most concentrations (excluding 5 and 12.5 g / l PAC). At the conclusion of the trial all concentrations had reduced shoot growth compared to the control.

It is not until 12 months through the trial that *Pistachia* shows significant differences in shoot length between concentrations of PAC become apparent, when only the control is significantly different from all other treatments (Figure 4.8) (Plate 4.9). At 18 months the difference is three tiered with the control significantly different from 12.5 and 25 g / l PAC which is then significantly different from 50 and 75 g / l PAC. In terms of application of injected PAC it would be more economical to use the lower of each of the non significant concentration pairs (i.e. 12.5 or 50 g / l PAC).

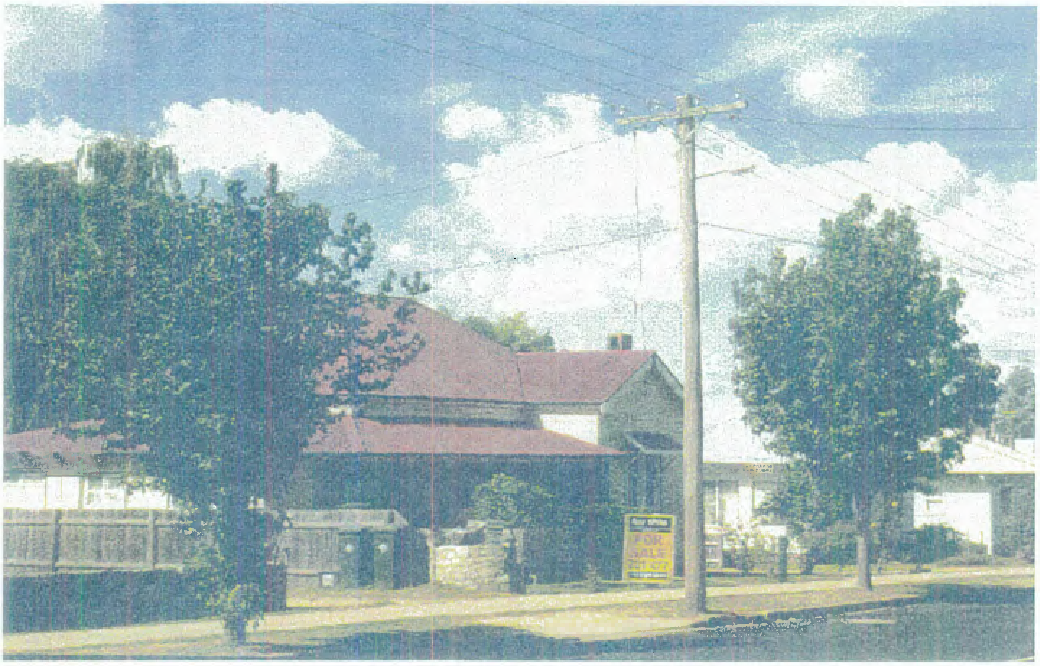
Relative to the other trial species, *Eucalyptus* was least responsive to injected PAC. Twelve months after injection with PAC there is no significant difference in shoot lengths between concentrations (Figure 4.6). At the conclusion of the trial the control was only significantly different from 50 g / l PAC which was then significantly different from 75 g / l PAC (Table 4.3).

The economic benefit from using PAC in a tree growth control program will be influenced by the response of individual species. From this trial we can conclude that *Liquidambar* and *Pistachia* can be controlled both economically and effectively using PAC. By contrast, even high concentrations (50 and 75 g / l) of PAC do not result in a significant reduction in shoot growth in *Eucalyptus nicholii*, to justify the expense of obtaining these formulations.

Phenology. The phenology of the species tested may also be considered in their overall response to PAC. Both *Liquidambar* and *Pistachia* are deciduous species whose growth period is restricted to a period of 7 months from October to April. During this period they must replenish diminished nutrients, produce leaves, flower, set seed or fruit and, acquire sufficient internal reserves to overwinter. By contrast, *Eucalyptus nicholii* is evergreen with an inherently low water potential and transpiration rate whose growth is not restricted to a period

Plate 4.7 Comparison of high (left) and low (right) PAC injected *Liquidambar* trees. Note the clustering of leaves and proliferation of epicormic shoots on the left specimen.

Plate 4.8 Detail of a specimen of *Liquidambar* treated with the highest concentration (50 g / l PAC). Note bunching of leaves, a result of reduced shoot growth.



in spring and summer. The deciduous species transport and utilise injected PAC over a shorter period of time than *Eucalyptus* . This might result in a sustained period of PAC activity in deciduous species year to year. Potential carryover of PAC from year to year in one of these species has been investigated (results not shown here) and it seems likely that it is occurring.

Compartmentalisation may also influence the effectiveness of PAC. In these trials this process was monitored from periodic observation of the drill hole sites within the trunk. Sealing with brown mastic compound appears to have reduced production of external wound tissue around the drill hole. Internal compartmentalisation (wounding) was not measured.

Transpiration rates in *E. nicholii* would have been lower than for the two deciduous species. *Eucalyptus* is more efficient in its use of water than either *Pistachia* or *Liquidambar*. Estimates of maximum total transpiration from leaves is between 1,500 - 3,000 µmol.H_2O.m.s in sclerophyllous plants such as *Eucalyptus* while it is slightly higher at 2,500 - 3,700 µmol.H_2O.m.s in light adapted deciduous plants such as *Liquidambar* and *Pistachia* (Larcher 1995).

4.6.2 Morphological response to PAC

The effects of PAC upon the overall morphology of trees was obvious. Shoots of high PAC concentration trees were visibly shorter than those of control or low PAC concentrations trees. Greatest differences were observed in *Liquidambar* (Plate 4.7) whereas the differences were not as pronounced in *Eucalyptus.*

In both deciduous species, while current shoots were retarded in their growth by PAC, new shoot production was extremely compact. This resulted in shoots developing as small spurs in *Liquidambar* (Plate 4.8) where the effect was most pronounced. In addition, these short shoots produced similar numbers of leaves to shoots of untreated trees giving them a 'tufted' appearance. The desirability of this characteristic is debatable.

As noted in other experiments, the leaves of *Liquidambar* developed a dark green colour and were more rigid in texture and feel than untreated tree leaves. The darkening colour of leaves was not observed in either *Pistachia* or *Eucalyptus*. In the case of *Eucalyptus*, the natural grey-green colouring of the leaves would mask any green colour.

The production of fruits in *Pistachia* (Plate 4.10) also appeared to increase in trees treated with higher concentrations of PAC . This is most likely due to resource partitioning favouring reproductive development over vegetative development. Increase in fruit set could also be an

Plate 4.9 A specimen of *Pistachia* treated with the highest concentration (75 g / l PAC). Some bunchiness of the leaves is evident.

Plate 4.10 Fruit from a tree of Pistachia treated with the highest concentration (75 g / l PAC).

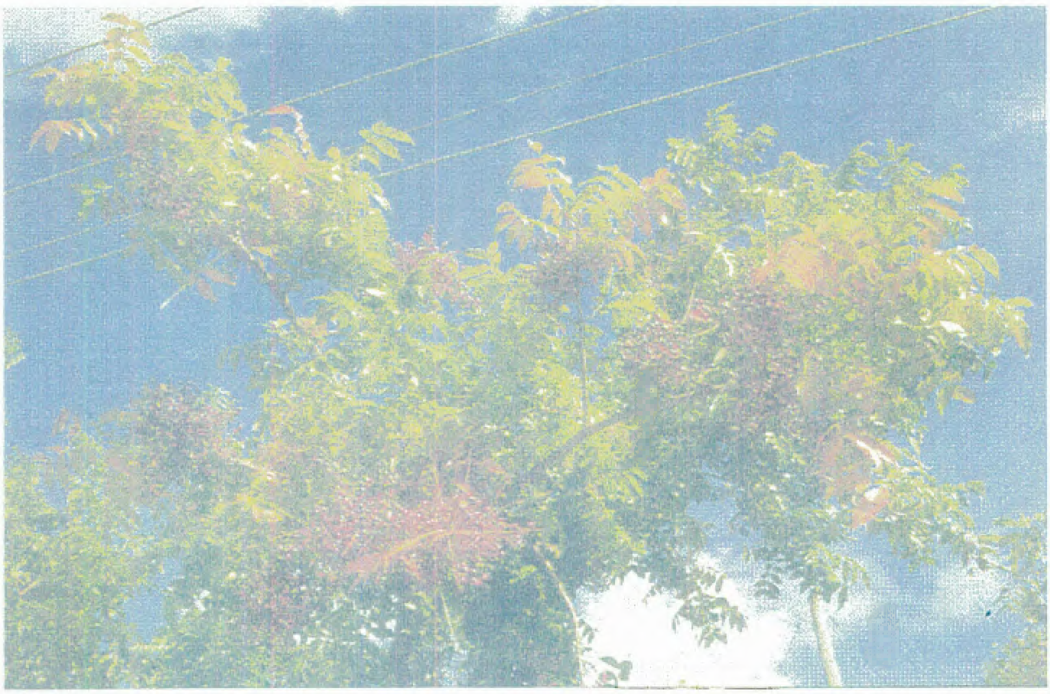


Plate 4.11 Detail of 'escape shoots' on a specimen of *Eucalyptus nicholii*

effect resulting from PAC induced synchronisation of flowering in treated male and female individuals as seen in *P. vera* (Porlingis 1993).

The production of 'escape shoots' by *Eucalyptus* (Plate 4.11) and *Pistachia* was notably restricted in high PAC concentration trees. Escape shoots arise from rapid growth in sections of the tree canopy. Resources are selectively partitioned into the growing shoot at the expense of lateral shoots resulting in a rapidly extending, whippy cane. These escape shoots pose a substantial threat to overhead powerlines owing to their rapid growth in a single season.

4.6.3 Environmental influences and response to PAC

Results of this trial also suggest that in addition to variation in response to PAC between species, there is variation in response related to local climatic differences. In common with trials in other locations the response of *Eucalyptus* varied site to site. Three of these are included for comparison.

To summarise my trial, PAC was shown to reduce shoot growth in *Eucalyptus nicholii* by up to 24% (at 75 g / l) compared to the control after 18 months, in a climate with mild to warm summers (mean max. Jan. temp. 26.7 °C), cold winters (mean min. July temp. 0.4 °C) and predominantly summer rainfall (780 mm / annum) (Figure 4.9b).

The first of the other studies was conducted by Griffin *et al.* (1993) in the Latrobe Valley, Victoria. In a site with mild summers (mean Jan. temp. 19 °C) and cold winters (mean July temp. 8.5 °C) and predominantly winter rainfall of about 800 mm / annum, trees of *Eucalyptus globulus* Labill. injected with 10 g / l PAC showed a reduction in shoot growth by around 85 %, compared to the control, over a period of 29 months.

The second study was conducted in South Australia, where similar injection trials were conducted in a climate with hot (mean max. Jan. temp. 27.9 °C), dry summers and mild winters (mean min. July temp. 6.8 °C), with predominantly winter rainfall of 450 mm / annum (Figure 4.9a). Using PAC at a concentration of only 20 g / l, the reduction in regrowth of *Eucalyptus odorata* Behr, *E. spathulata* Hook. and *E. camaldulensis* Dehnh. was 80 %, 40 % and 70 % respectively after 24 months, compared to the control (Deans 1989).

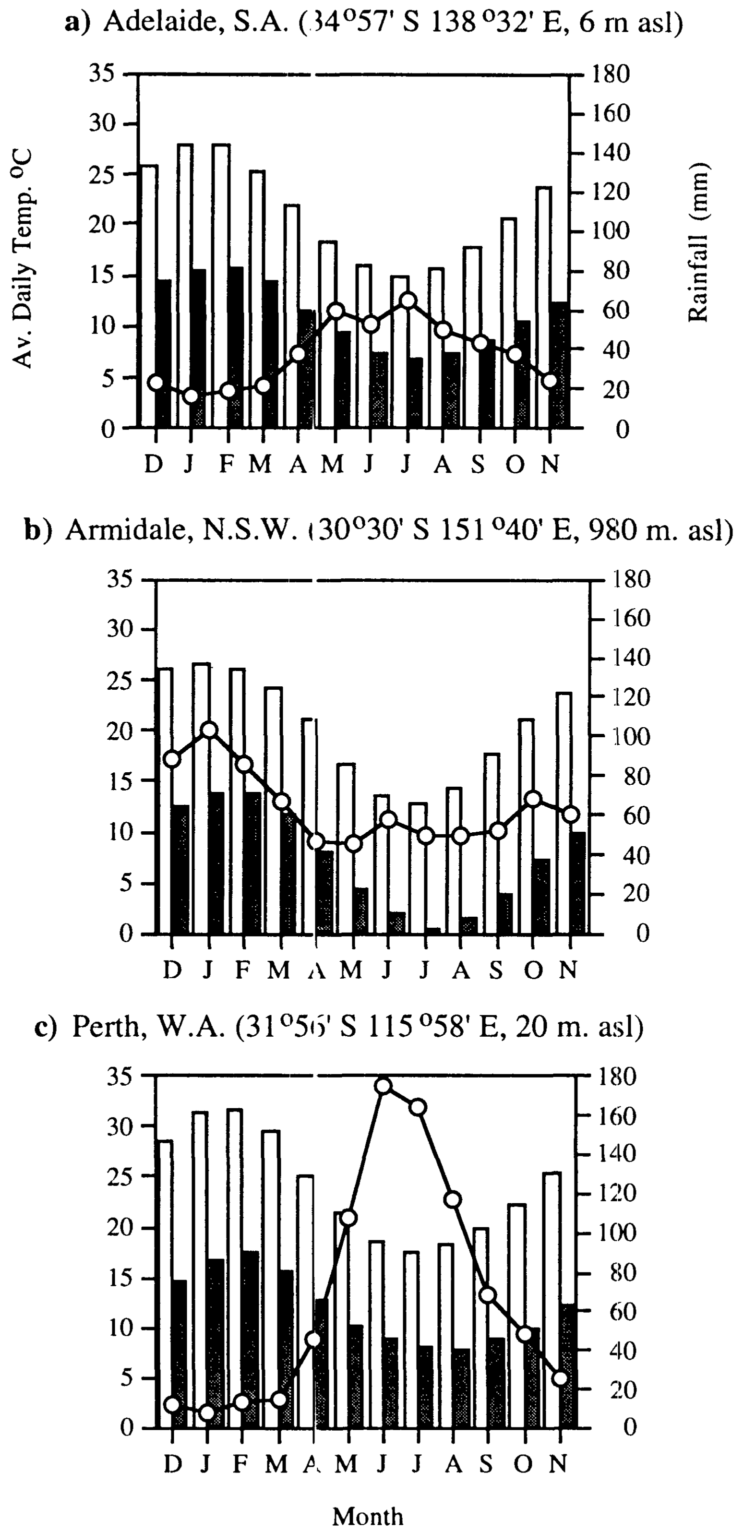


Figure 4.9 Temperature and rainfall data for three Australian locations: **a)** Adelaide, **b)** Armidale and, **c)** Perth. Unshaded bars represent maxima, shaded bars; minima. Data from Bureau of Meteorology (http: //www.bom.gov.au). Circled line represents rainfall.

The final study was conducted in Western Australia (mean max. Jan. temp. 31.4 °C, mean min. July temp. 8.6 °C, 800 mm rainfall, winter dominant (Figure 4.9c)) using 20 g / l PAC resulted in a reduction in shoot growth of up tc 70 % after only 12 months (Cox 1990).

The variation in response between the four climates in terms of growth reduction for *Eucalyptus* is dramatic. Under local conditions the reduction in growth was comparatively less, even when the concentration of PAC injected was three-fold higher. We could infer from this comparison that some trees in cooler climates may require higher concentrations of injected PAC to elicit a response. And, that this variability in response is most likely related to temperature and rainfall distribution.

4.6.4 Anatomical response to PAC

There is also some speculation in relation to the influences that the anatomy and physiology of each species has upon PAC uptake and movement within species.

For example, it is recognised that the velocity of the transpiration stream is higher in ring porous species than in diffuse porous species (Huber 1935). Since PAC is injected into the transpiration stream its subsequent movement will be influenced by sap flow. As with many deciduous species, both *Liquidambar* and *Pistachia chinensis* are ring porous suggesting a rapid transpiration stream. By contrast, *Eucalyptus* is diffuse porous, having by inference, a slower transpiration stream even independent of climatic considerations. This difference in anatomy could explain the poorer response in *Eucalyptus*.

Likewise the wood grain, which was not investigated in this study, could also be a contributing factor in the poor response in *Eucalyptus*. Field observations made during the trial revealed that in *Liquidambar*, the degree of growth inhibition as correlated to leaf size reduction was greatest in those branches immediately above the injection sites. This would suggest that *Liquidambar* has straight grain wood. Neither *Pistachia* nor *Eucalyptus* showed such localised effects.

4.6.5 Conclusions

PAC is an effective tree growth regulator in the cool climate of the Northern Tablelands. From this study, we are able to recommend optimal concentrations of PAC to achieve the greatest shoot reduction under local conditions. These recommendations may also be applicable to similar species in other Australian or international homoclimes.

The cost effectiveness of this control requires investigation although it is expected that this would, at best, be marginal for *Eucalyptus nicholii.* Species must be treated on a case-by-case basis and this treatment is influenced by the prevailing local climatic conditions. A number of other recommendations can also be made with regard to methodology in application of PAC.

The first of these involves the concentration and volume of PAC used to inhibit shoot growth. *Eucalyptus nicholii* was the poorest respondent to trunk injection of PAC. Even at the highest concentration (75 g / l) the inhibition of shoot growth was only marginally better than for lower concentrations. The 75 g / l PAC concentration is the highest attainable in ethanol solvent. In early years of experimentation with trunk injected PAC the compound had been dissolved in methanol. This solvent was found to be unsuitable to many trees, causing phytotoxic symptoms shortly following injection. Therefore, we cannot alter the solvent to create PAC solutions in higher concentrations. Nor can we increase the concentration of PAC without it crystallising out of solution prior to injection. There are three main solutions to overcome this problem; a) increase the number of injection holes, b) the volume injected into each hole or, c) improve our injection methodology and delivery of PAC.

Increasing the number of injection holes would deliver a greater volume of compound to the tree. The problem is that the size of the tree and its trunk may preclude more closely spaced injection holes. The currently recommended minimum distance for injection hole spacing is 15 cm. Any closer than this runs the risk of mechanical breakdown of the trunk between the injection sites causing bark blowout. In trees of sufficient size to accommodate more holes the problem arises that for each increase in trunk circumference (on which drill hole spacing is currently based), there is likely to be a doubling or trebling of tree volume. Where volume greatly exceeds trunk circumference the delivery of PAC would reach a ceiling beyond which extra injection sites are ineffectual.

The second option is to increase the volume injected into each hole. As with increasing the number of holes, the mechanical ability of the wood to resist weakening and destruction will be a key factor. Denser woods may be able to accommodate the higher pressures generated locally wound injection sites if volumes were increased. Likewise, a rapidly transpiring tree could relieve localised pressure caused by injection much faster than a slowly transpiring specimen.

The improvement of our injection methodology and delivery of PAC would appear to be the most promising approach. To do this we must acquire data concerning the tree's wood characteristics, its physiological processes (e.g. transpirational losses, sap flow) and assess the local climatic conditions surrounding the time of injection. Each of these factors has already been discussed in greater detail in this chapter.

The other area of improvement lies with injection technology. Presently, there are two approaches by which to introduce compounds into trees via trunk injection; passive and active. Passive mechanisms rely on gravity feeding of materials. Active mechanisms use positive pressures to introduce compounds.

The degree of pressure required will be dependent on the factors previously outlined. Low pressure can be just as effective as high pressure and is likely to cause less mechanical damage to the tree. Another alternative, in its early experimental stage, is the use of negative pressure to introduce compounds. This new method for introduction of trunk injected compounds is presented in Chapter 6.

The final question that needs to be resolved is why PAC does not affect all species equally (i.e. why are some species not responsive to PAC) This issue is examined in the final section of this discussion.

4.7 Some theories on species which are non-responsive to PAC

4.7.1 Introduction

The issue of why particular species of tree do not respond to applications of PAC has not been addressed by the manufacturers or utilisers of this compound. And yet, it is one of great theoretical and practical importance.

Lack of research into this area has probably been due as much to the drive to find plants which do respond to PAC as to a general lack of interest in its investigation. Similarly, research into the breakdown or degradation of PAC both within the tree and within the soil has attracted little interest. To my knowledge there is no work which directly addresses this question with clear chemical terms. This section intends to address the question of non-responsiveness of species to PAC.

Three theories are proposed which could individually, or collectively, account for the lack of response seen in many species treated with PAC. These fall into; i) rapid growth, ii) physiological overproduction of sterols and, iii) presence of PAC degrading bacteria *in vivo*. Each theory is supported by available evidence where appropriate.

4.7.2 Rapid growth and sequestering of PAC

The speed with which any tree will lay down secondary tissue is dependent primarily on its growth environment. Where sufficient moisture is available and temperatures are moderately high (28 - 35 °C) trees can dramatically increase length and gain volume in relatively short periods of time. These conditions are best exemplified in sub-tropical, coastal climates where there is no distinct wet and dry season.

In hot conditions trees transpire vast quantities of moisture. In most situations, soil moisture deficits translate into drought stress within a tree with the result that stomatal closure minimises moisture losses. Where moisture is readily available the tree continues transpiring and growing until another factor limits its growth (e.g. nutrients, light, pH).

If PAC is injected into a tree in this 'ideal' situation the immediate effect is that the compound will be rapidly transported to the canopy by the transpiration stream. With a rapidly flowing transpiration stream present, crystallisation of the compound out of solution may not occur close to the injection point, but possibly further up the trunk of the tree. The temperature of the stream itself will also influence the solubility of PAC within it. Slight increases in temperature would be sufficient to allow a greater portion of the compound to remain in solution. This portion would move rapidly to the shoots and leaves leaving no 'reservoir' lower down within the tree. The portion of material crystallised out will also be more readily eluted over time if the transpiration stream is both rapid and warmer.

These conditions may help to explain why many tropical species growing in coastal, sub-tropical environments require very high concentrations of PAC to elicit a response or, do not respond at all. For example, *Jacaranda mimosaefolia* Juss (Jacaranda) and *Flindersia sp.* R.Br. are two species requiring very high levels of PAC to elicit a response (McDonald 1992). These trees grow on the north coast of New South Wales, Australia where temperature is equable (mean min. 12-15 °C, mean max. 24-27 °C) and rainfall is frequent (mean average 100 mm / month).

Injected PAC is initially distributed near the injection hole if it is not rapidly transported to the shoots and leaves. Rapidly growing trees will be producing new secondary xylem and phloem increasing the girth of the plant. If PAC is initially deposited in the functional xylem it may be quickly sequestered as newer xylem is produced, while the xylem in which it was deposited becomes non-functional.

I have presented two possibilities to explain why PAC is not effective in particular types of trees. What should also be addressed is the issue of trunk anatomy as related to introduction and distribution of PAC. However, it is beyond the scope of this thesis to examine this aspect, although it has been touched upon in Chapter 4.

4.7.3 Sterol production *in vivo*

With such a potentially enormous number of compounds synthesised, utilised or extracted from plants and other organisms, it would seem unusual were there not a few which inactivated triazole compounds.

This 'inactivation' could assume a number of forms from direct conjugation to degradation or, interference at the binding site of PAC with cytochrome P-450.

PAC is known to have both plant growth retarding and fungicidal properties. The fungicidal properties relate to its basis as a triazole compound. Triazoles, along with 1-substituted imidazoles are known to inhibit biosynthesis of ergosterol, the principal sterol (steroid alcohol) in most fungi and the essential component of fungal membranes (Koller 1987).

Were high levels of sterols present in the plant system they could compete with PAC for binding sites with cytochrome P-450. The result would be that PAC would not have an opportunity to bind with cytochrome P-450 and then inhibit the GA biosynthesis pathway. Within higher plants there are sterols including stigmasterol, sitosterol, and the aglycone portion of saponin is a sterol. Whether this competitive binding alters GA biosynthesis, and what is the fate of PAC, if it cannot fulfil its biochemical function, is also speculative.

4.7.4 PAC degradation by bacteria *in vivo*

Eubacteria are an enormously diverse group of bacteria that share many environments with other plants and animals. They also contain almost every variety and combination of biochemical energy extraction that is thought to be feasible on the basis of the molecular composition of the biosphere (Knox *et al.* 1995). There are some genera of eubacteria which also degrade PAC.

PAC utilising microorganisms were enriched from eight agricultural soils in Tasmania. Nine bacterial isolates capable of PAC degradation were isolated, eight belonging to the genus *Pseudomonas* and one to the genus *Alcaligenes* (Jackson *et al.* 1996). The half-life of ^{14}C-PAC with the *Pseudomonas* isolate in batch culture was approximately 13 d.

Both *Pseudomonas* and *Alcaligenes* have been found in vase water with cut stems of *Rosa hybrida* L. cv. Sonia (de Witte and van Doorn 1988, van Doorn *et al.* 1991). These bacteria had apparently entered the xylem through the freshly cut surfaces. Debate as to whether these bacteria are present within the plant prior to cutting is unresolved (Williamson pers. comm.).

There is a possibility here *Pseudomonas* or *Alcaligenes* could enter the plant through wound sites and colonise sites within the xylem vessels. PAC degrading strains of these bacteria may have an influence on the effectiveness of the compound within the plant. The degradation of PAC by *Pseudomonas* is fairly rapid and could certainly take place before the compound was able to completely affect GA biosynthesis inhibition.

4.7.5 Conclusions

This section has presented three ideas on which to base future work with PAC in non-responsive plant species. However, there are still many questions which remain unresolved. The intrinsic mechanism by which PAC inhibits GA biosynthesis has been studied but not elucidated.

For example, does each molecule of PAC function only once in blocking GA biosynthesis, or can a single molecule affect a number of reactions? How dependent is this inhibition on the presence of cytochrome P-450? Would an alteration in levels of cytochrome P-450, sterol production or bacteria *in vivo* affect the degree of inhibition possible? We do not know the answers to these questions and they would prove useful areas of research in helping to make PAC a more universally effective compound for a range of plants.