Chapter One

General Introduction

The word 'hormone' is often thought of as only being relevant to humans or animals. People rarely consider that plants should have (or perhaps even need) such things.

Plant hormones take a back seat to animal hormones because they are not as seemingly obvious in their effects but they influence seed germination, cell elongation and expansion, flowering and fruiting. They are enormously important substances. Without them, plants would not grow, develop, differentiate, mature, and produce offspring. In essence, without hormones, plant life would cease to exist.

Plant growth regulators are synthetic compounds, unlike the naturally occurring plant hormones. They are in common use whether people know it or not. Gardeners who use 'hormone' powder are using a synthetic plant growth regulator to encourage initiation of roots on cuttings while the derivatives of 2,4-D, often used as weedkillers, are nothing more than powerful growth inhibitors. Thus, plant growth regulators and inhibitors are used on a daily basis without most people realising they are dealing with them.

One plant growth inhibitor is paclobutrazol (PAC), whose range of uses and effects cover the areas of agronomic, floricultural and horticultural research. This thesis examines PAC in particular detail.

The aims of this thesis are to;

* summarise and consolidate the literature regarding plant hormones, regulators and inhibitors,
* introduce the plant growth regulator, PAC,
* investigate the detection of PAC both *in vitro* and *in vivo*,
* investigate the effectiveness of PAC in controlling growth of street trees under powerlines,
* present new evidence concerning the movement of PAC *in vivo*,
* present an alternative method to trunk injection for introducing PAC into trees.

The area of plant growth hormone and regulator research has been active since the discovery of the existence of auxin as early as 1880. Since then, several classes of naturally occurring plant hormones have been discovered. Concomitant with this research has been work developing synthetic compounds which manipulate plant growth. Examples include dwarfing crops to prevent lodging or increasing flowering or fruiting for greater yields. Chapter 2 examines the literature encompassing the breadth of plant growth hormones, regulators and inhibitors with particular emphasis placed on the properties and effects of the plant growth inhibitor, paclobutrazol (PAC).

Having established PAC as the main focus of this thesis, initial experimental work with this compound is presented. Chapter 3 introduces some baseline studies with PAC investigating its reactivity and simple detection. These investigations lead to experimentation with more complex techniques in an attempt to find a rapid and efficient method for the detection of PAC both *in vitro* and *in vivo*.

Having highlighted the extensive applications and effects of PAC, the possibility for the utilisation of this compound in the control of growth of trees under powerlines is presented. Chapter 4 is comprised of a field trial study, using PAC to inhibit growth of street trees under powerlines. PAC's effectiveness is assessed in light of plant anatomy, environmental conditions and physiology. Recommendations are made as to the usefulness of using PAC as a growth control option in contrast to annual pruning of trees.

While it is accepted that if PAC is injected into the xylem it will affect the tree, the basic movement of PAC *in vivo* has not been fully investigated. Another possibility for translocation of PAC is through the phloem system of the tree. Chapter 5 presents experimental work which contradicts research that PAC is exclusively xylem mobile within plants. PAC is also phloem mobile, and this opens up questions about its actual translocation within plants.

Having established that PAC might also be phloem mobile in some species we need to also address the question of the relative efficiency of the current method of PAC delivery into the tree. Most PAC is trunk injected, but is this really the most efficient and safe method of delivering PAC into the tree system? Chapter 6 presents an alternative method to trunk injection for introducing compounds, such as PAC, into living trees. Known as negative suction, it is a remarkably simple approach, and has proven very promising based on my experimental work.

Finally, Chapter 7 summarises the experimental work presented in this thesis, highlighting the main issues which have been resolved by this research. Possibilities for future research are also discussed.

Chapter Two

Literature Review

1. History of Plant Hormones

1.1 General Introduction

The concept of plant hormones is originally credited to Sachs (1874). He postulated the existence of organ-forming substances controlling growth and development. This theory was not widely accepted at the time as Sachs's methods were extremely crude and did not show distinct differences between these organs and the compounds they contained.

The American Society of Plant Physiologists published the nomenclature of chemical plant regulators in 1954. They defined plant hormones as follows:

'...regulators produced by plants, which in low concentrations regulate plant physiological processes. Hormones usually move within the plant from a site of production to a site of action'.

While this may be one of the earliest attempts to specify the nature of a plant hormone this definition is not immutable. For example, Salisbury and Ross (1985) state, 'Most plant physiologists accept a definition that is similar to that developed for animal hormones, even though there is no evidence that the fundamental biochemical actions of plant and animal hormones are the same'. Their definition rests as 'A plant hormone is an organic compound synthesised in one part of a plant and translocated to another part where, in very low concentrations, it causes a physiological response'.

Plant hormones differ from animal hormones in that they are not produced in specific organs, nor have highly specific effects (Hill 1980). Hill continues by providing another definition, 'a plant growth substance (or plant hormone) is an organic substance which is produced within a plant and which will, at low concentrations, promote, inhibit or qualitatively modify growth, usually at a site other than its place of origin. Its effect does not depend upon its calorific value or its content of essential elements'. The term 'plant growth substance' was suggested by Trewavas (1981).

Thus the terms plant hormones, plant growth substances and plant growth regulators have all been used in an *ad hoc* fashion. For the purposes of consistency this project will use the modern and concise definition stated by Davies (1995): 'Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations'. This is very general definition by design because the active concentrations and sites of production and utilisation of plant hormones is variable.

Luckwill (1981), in a comprehensive breakdown of types of plant growth regulators, divides them into nine groups. These are ;

I. natural hormones
II. ethylene releasing agents
III. hormone transport inhibitors
IV. hormone mimics
V. hormone antagonists
VI. growth retardants
VII. growth inhibitors
VIII. defoliants desiccants and ripeners
IX. other types.

The intrinsic difference between growth retardants and growth inhibitors is that the former affect the sub-apical meristem and the latter, the apical meristem. This division is useful and shall be utilised throughout this review.

1.1.1 Auxins

The first specific plant hormone to be appreciated was probably an auxin. This was recognised by Darwin in 1880 in his empirical work published under the title 'The Power of Movement of Plants'. He described an experiment in which phototropism of coleoptiles of *Phalaris canariensis* was examined. If an etiolated coleoptile is exposed to a directed light source it undergoes a curvature towards the source. When he covered the coleoptile tip with a tinfoil cap and it is placed in darkness with the lower portion illuminated in the same manner there was no curvature. His conclusion was 'that when seedlings are freely exposed to a lateral light, some influence is transmitted from the upper part to the lower part, causing the latter to bend' (Darwin 1880). This work was confirmed by Boysen-Jensen (1913) in experiments with coleoptiles of *Avena sativa* , and also by Paal (1914, 1919). Both experimenters induced curvatures in decapitated plants coleoptiles by using excised, photostimulated tips from other test

plants. They showed there was a substance which could diffuse from stimulated tip to decapitated tip and cause curvature.

In 1928, Went extracted an active substance from *Avena* coleoptile (Roberts 1988). He placed a photostimulated, excised coleoptile tip on an agar block. This block was placed on one side of the stump of an unlit, decapitated coleoptile. The coleoptile curved away from the site of the agar block. This was called the '*Avena* Coleoptile Curvature Test' and is still used as a bioassay for auxins.

The term auxin is derived from the Greek *auxein* meaning 'to grow' and was proposed by Kögl, Haagen-Smit and Went to designate a particular substance which had the property of promoting curvature in the *Avena* Coleoptile Curvature Test (Moore 1979).

Auxin was not purified and chemically identified until the early 1930s. Kogl (1931) obtained auxentriolic acid from human urine. In 1933, Kogl analysed urine again and found substances called 'auxins A and B' and 'hetero-auxin'. The structures of these were postulated on the basis of their chemical degradation. 'Hetero-auxin' was found to be identical in structure to indole-3-acetic acid (IAA). Not until the work of Haagen-Smit (1946) was IAA isolated from higher plants.

Following introduction of detection using paper chromatography (Bennet-Clark 1951), auxins were found to be widespread in higher plants.

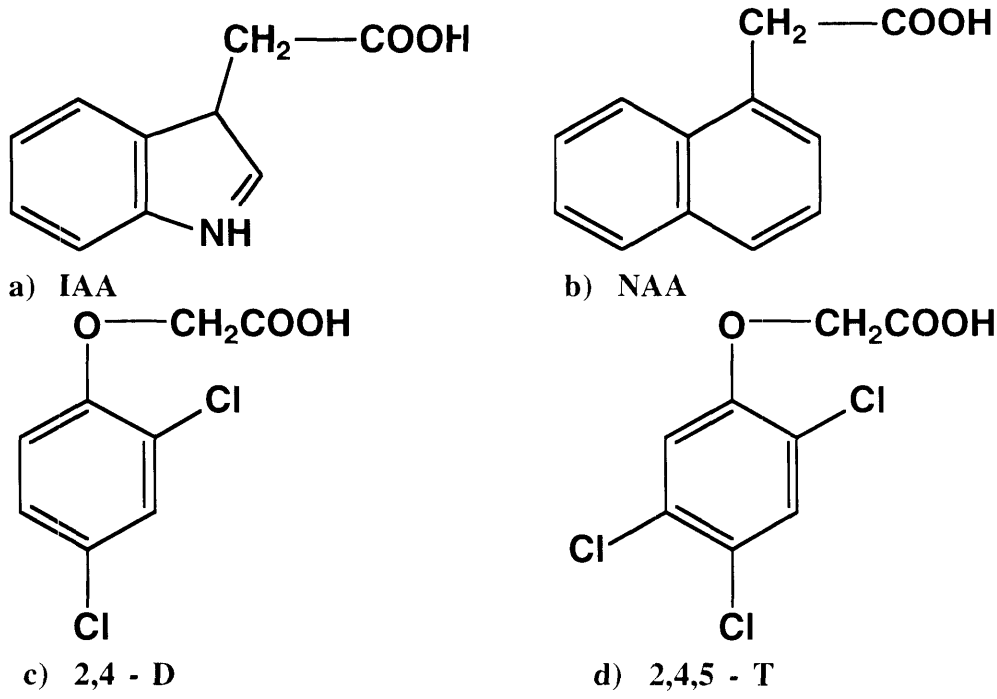


Figure 2.1 Chemical structure of some naturally occurring and synthetic auxins

IAA (see Figure 2.1.a) is the major auxin in most plants. Other compounds may display weak auxin activity (e.g. phenylacetic acid, indoleacetaldehyde, 4-chloro-IAA) (Davies 1995). In addition, synthetic auxins have been produced as promoters of root initiation (napthaleneacetic acid - NAA (Figure 2.1.b) and indole butyric acid - IBA) or as herbicides (dicholorophenoxyacetic acid - 2,4-D (Figure 2.1.c) and 2,4,5 - trichlorophenoxyacetic acid - 2,4,5-T (Figure 2.1.d)).

1.1.2 Gibberellins

Gibberellins were originally extracted from the fungal ascomycete *Gibberella fujikuroi* (imperfect stage; *Fusarium moniliforme*) following observations that rice plants infected with this fungus grew taller than uninfected plants and suffered leaf chlorosis and wilting.

Kurosawa (1926) recognised that the fungus produced a 'toxin'. Culturing the fungus on rice extracts he then grew seedlings and supplied them with aqueous extracts of culture filtrate of the fungus. Plants developed symptoms previously observed in the field.

Using culture filtrates, Yabuta (1938) isolated two active compounds named gibberellin A and gibberellin B. The name gibberellin was assigned by Yabuta and Sumiki, and recognises the compound's origins. Research into gibberellins was discontinued during World War Two.

Following World War Two, three groups emerged to research gibberellin intensively: the University of Tokyo group, the ICI group in Great Britain and the Northern Regional Research Laboratory (NRRL) in the U.S. Successive isolations and characterisations of gibberellins followed.

In 1954, the ICI group isolated an active substance which they named gibberellic acid (Curtis and Cross 1954). In 1955, the NRRL reported separation of gibberellin A into two components - gibberellin X and gibberellin A. Also in 1955 the University of Tokyo group separated their gibberellin A into three gibberellins - gibberellin A_1, A_2 and A_3. Comparison revealed that gibberellin A and gibberellin A_1 were identical as were gibberellic acid, gibberellin X and gibberellin A_3. Gibberellin A_2 was actually a new gibberellin. Gibberellic acid (a.k.a. gibberellin X and gibberellin A_3) became known as GA_3.

To this point gibberellins were only known from extracts of a fungus. In 1956, West and Phinney (1956), and Radley (1956), independently determined that gibberellins were natural products of higher plants.

Numbers of isolated and characterised gibberellins increased rapidly over the subsequent years. Each is assigned an 'A number' in more or less the order they are discovered. In 1979, the number of GAs from all sources was 52 (Moore 1979). In 1986, this was at 68 (Takahashi *et al.* 1986), and in 1995 had increased to 89 (Sponsel 1995). In 1996 Mander and Cwen had discovered a new GA from spinach plants ; GA_{99}.

A gibberellin has been defined as a compound having an *ent*-gibberellane skeleton and biological activity in stimulating cell division or cell elongation, or both, or such biological activity as may be specifically associated with this type of naturally occurring substance (Paleg 1965) (Figure 2.2).

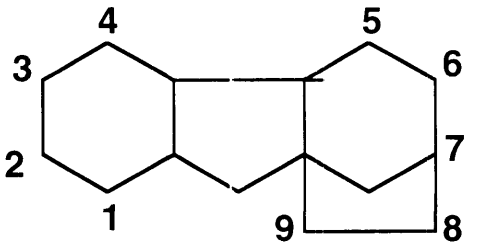


Figure 2.2 The gibbane carbon skeleton which is the basic structure of gibberellic acid and other compounds with gibberellin-like activity

1.1.3 Cytokinin

The existence of 'cell division factors' was suggested by Weisner (1892) but not experimentally proven to exist until Haberlandt (1913) induced division of cells in potato tubers with phloem diffusates.

Jablonski and Skoog (1954) found that tissue cultures of tobacco stem pith would undergo cell elongation but not division on a basal medium with auxin. If extracts containing coconut milk, vascular tissue, malt or autoclaved DNA were added, cell division was obtained .

Miller *et al.* (1955) identified the first active cell division promoter when they isolated 6-(furfurylamino) purine from autoclaved herring sperm DNA. This compound was

called kinetin and though biologically active is not naturally occurring in plants as it is a by-product of heating DNA.

Originally called kinin, the term cytokinin was introduced to distinguish it from a group of materials of animal origin. Cytokinin has come to be defined as 'a compound which, in the presence of optimal auxin, induces cell division in tobacco pith or similar cultures, and in its other activities also resembles kinetin' (Letham 1978).

In 1963, Letham had isolated in crystalline form, a natural cytokinin from immature corn kernels which he called zeatin (Figure 2.3). Zeatin was thought responsible for the cell division induced from coconut milk extract. Following this announcement the number of known, naturally occurring cytokinins increased. By 1967, the cytokinin containing extracts had been prepared from approximately 40 species of higher plants, and it is now confidently assumed that cytokinins are ubiquitous among seed plants and perhaps throughout the plant kingdom (Moore 1979).

CPPU (N - (2 - chloro - 4 - pyridyl) - N' - phenylurea) is one synthetic cytokinin-like substance Takhashi *et al.* (1978) used to stimulate fruit growth of apple Sansavini *et al.* (1990) and kiwifruit Biasi *et al.* (1991). CPPU promotes cell division and increases fruit size when applied during the first stages of fruit development.

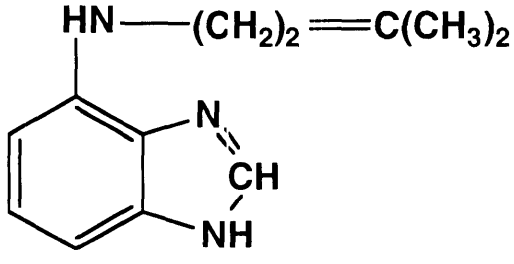


Figure 2.3 Chemical structure of zeatin, a natural cytokinin

2. History of Plant Growth Regulators

2.1 General Introduction

The difference between plant hormone and plant growth regulator (PGR) should be addressed from the outset. The definition of a plant hormone as proposed by Davies (1995) has already been presented (see 'History of Plant Hormones') while a PGR may be defined as;

'a synthetically created compound from inorganic or organic sources which, when applied exogenously to a plant, induces a physiological response'.

Included in this group for convenience are compounds known as 'growth retardants' whose function is to reduce or inhibit growth of plant organs. Dicks (1979) defined growth retardants as;

'organic compound, which, when applied to a responsive plant, reduce(s) the rate of stem elongation by inhibiting subapical meristem activity, normally without exerting substantial effects on leaf production and development or inducing other growth malformations'.

In the process of performing this function other physiological and morphological changes may manifest themselves which are not intrinsically deleterious to the survival of the plant.

According to Gianfagna (1995) PGRs have been important components in agricultural production even prior to the identification of plant hormones. Many growth regulators have been trialled and tested since the 1950s and no single database provides a complete listing. The process by which a compound progresses from laboratory to commercial release is outlined below.

Chemical companies who first discover a compound will release it for trial under a **code number** (e.g. PP 333, BAS 111..W, LAB 198 199). Once a patent has been obtained for the compound a **chemical name** is assigned. The chemical names are often extremely long and usually shortened to an **acronym** or the compound given a **trivial name** (e.g. chlorocholine chloride for 2 - chloroethyltrimethylammonium chloride) which is then shortened to an acronym (e.g. CCC). A compound which is successfully patented and formulated for marketing and commercial sale is then given a

trade name. If the growth regulator becomes widely used it is given a **common name** which when passed by the ISO (International Standards Organisation) is the name used for the compound in scientific publications (Luckwill 1981).

Synonyms associated with growth regulators often cause confusion which can arise when trivial names have been misused or code numbers used instead of the accepted trade name. As a result, the listing below cannot be declared a comprehensive summary.

2.1.1 Maleic hydrazide

One of the earliest releases of a PGR was **maleic hydrazide** (syn. MH-30®, Slo-Gro® ; 1,2 - dihydro - 3.6 - pyradazinedione) by the U.S. Rubber Company in 1948 (Figure 2.4). This compound is classed in Group VII (Luckwill 1981). MH-30 found application in the tobacco industry to control suckering, to prevent sprouting in onions and potatoes and retard growth of turfgrass. As an analogue of the pyrimidine base, uracil, the compound inhibits cell division by reducing nucleic acid biosynthesis in roots and shoots and is a hydrazine derivative. In addition, MH has been reported as having anti-auxin activity (Fox and Bullock 1951).

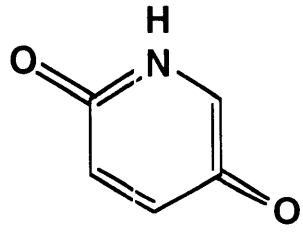


Figure 2.4 Chemical structure of maleic hydrazide

MH is recorded as retarding growth in red maple, elm, weeping willow (Rai and Hamner 1955), sycamore (*Platanus* spp.), poplar (*Populus* spp.), *Eucalyptus* (Ellis 1964) and *Pinus* spp. (Sachs *et al.* 1975a). Tinus (1974) found it was not effective against green ash (*Fraxinus pennysylvanica* Marshall). At high concentrations MH is phytotoxic and since this early work compounds with less potentially harmful effects have been developed resulting in a decrease in interest in MH.

A number of other PGRs were also developed during the 1950s (propham, chloropropham) including several cotton defoliants such as S,S,S - tributyl phosphorotithioate.

2.1.2 Chlormequat

In 1959 the American Cyanamid Company introduced Chlormequat (syn. Cyocel® ; CCC - 2 - chloroethyltrimethylammonium chloride) (Figure 2.5). This compound is classed in Group VI (Luckwill 1981). CCC was the first synthetic anti-gibberellin and is a quaternary ammonium growth regulator recognised by Tolbert (1960). The compound inhibits GA biosynthesis at a point beyond the formation of kaurene. Main use is in the prevention of lodging in wheat and oats by shortening and thickening the lower internodes of the stem. Tillering is also stimulated in treated plants.

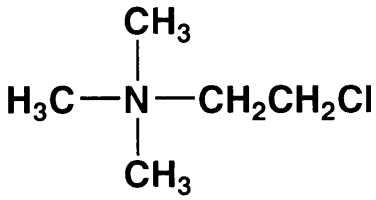


Figure 2.5 Chemical structure of chlormequat

Use of CCC as a tree growth regulator has had limited success and research is ambiguous as to its effectiveness. CCC failed to induce a significant response in most coniferous species tested including Arizona cypress (*Cupressus arizonica* E. Greene). Douglas fir (*Psuedotsuga menziesii* (Mirbel) Franco), Coulter pine (syn. Big-cone pine ; *Pinus coulteri* D. Don) (Pharis *et al.* 1967) and Eastern Hemlock (*Tsuga canadensis*(L.)) (Tinus 1974). Cathey (1975) did find that CCC retarded growth of maple, oak and sycamore. American elm (*Ulmus americana* L.) (USDA 1976) and green ash (Tinus 1974) were not affected by the compound.

2.1.3 Daminozide

Daminozide (syn. Alar®, B-9®, SADH ; N,N - dimethylamino - succinamic acid) was released in 1962 by Uniroyal Inc. (Figure 2.6). The compound is a derivative of hydrazine (cf. Maleic Hydrazide) that has found use as a growth retardant (Group VI). The compound causes internode reduction by reducing the rate of transverse division of cells in the subapical meristem (Sachs *et al.* 1960, Sachs and Kofranek 1963). Leaf production and arrangement is unaffected. It has been used successfully in controlling vegetative growth of fruit trees and promote manageable fruit set. Rademacher (1992) has since described the mode of action of acylcyclohexanediones (e.g. cimectacarb - CGA 163'935 and LAB 198 199) as being similar to daminozide. This compound blocks the conversion of biologically inactive GA_{20} into biologically active GA_1 via 3ß-hydroxylation. Daminozide is understood to behave in a similar manner.

Daminozide as the preparation Alar® retards stem elongation in maple, birch, *Catalpa bignonioides* Walt., sycamore and elm (*Ulmus* spp.) (Cathey 1975). Several coniferous species have been controlled using SADH including Arizona cypress, coastal redwood, coulter pine and *Pinus* spp. (Pharis *et al.* 1965, Sachs *et al.* 1967).

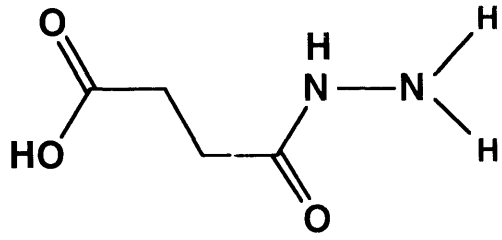


Figure 2.6 Chemical structure of daminozide

Control for *Eucalyptus* is ambiguous with Sachs *et al.* (1967) reporting a response in the field but this has not been repeated in glasshouse experiments (USDA 1976). Daminozide also significantly increased blossom coverage when applied as a foliar application to lowbush blueberry (*Vaccinium angustifolium* Ait.) (Lewis and Ju 1993).

2.1.4. Chlorflurenol

Chlorflurenol (chlorflurecol, flurenol, Maintain® ; Figure 2.7) belongs to a group of synthetic compounds known as 'morphactins'. Chlorflurenol is the 2-chloro derivative of flurenol or 9 - hydroxyfluorene - (9) - carboxylic acid methyl ester and is a Group III compound.

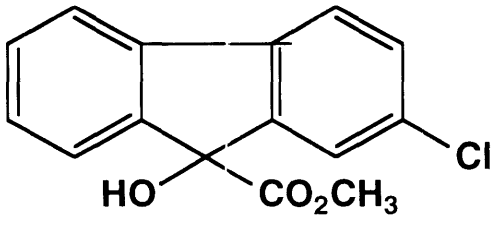


Figure 2.7 Chemical structure of chlorflurecol (Maintain 125)

The compound blocks the movement of endogenous hormones (*auxin*) which causes local accumulations (Luckwill 1981). They were originally called 'anti-gibberellins' without actual evidence of competitive inhibition (Krelle and Libbert 1967). There is evidence that morphactins are transported acropetally and basipetally and promote lateral branching and that their activity in the plant is often short-lived. Despite their high level of activity none of this group have found widespread commercial use (Lawrence 1984). This is probably due to their extreme influence on tropic responses

(phototropism, geotropism) and severe inhibition of apical dominance often resulting in unsightly, 'stunted' plants.

2.1.5 Ancymidol

The next major PGR introduction was **ancymidol** (syn. A-Rest® ; alpha - cycloprop - 4 - methoxy - alpha - [pyrmidin - 5 - yl] benzyl alcohol) by Elanco Products in 1971 (Figure 2.8). Another inhibitor of GA biosynthesis (Group VI), ancymidol blocks the conversion of kaurene to kaurenoic acid and belongs to a group of compounds known as pyrimidine methanols. Ancymidol has been used extensively in floricultural situations to produce shorter, more rigid stems. Secondary effect noted in potted plants are the production of darker green foliage and a delay in onset of flowering.

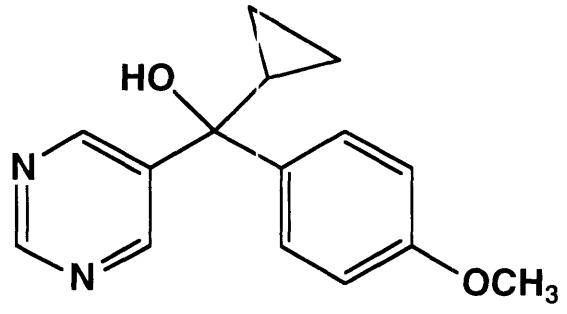


Figure 2.8 Chemical structure of ancymidol

Ancymidol as A-Rest® has been used to control maple, birch, *Catalpa bignonioides*, dogwood, elm and sycamore. The compound was not effective against magnolia (*Magnolia* spp.), crabapple (*Malus* spp.) and some species of *Pinus* (Cathey 1975).

Ancymidol has been known to become partly inactivated in media where pine bark is present due to possible binding mechanism between the compound and bark (Barrett 1982).

2.1.6 Chlorphonium chloride

Chlorphonium chloride (syn Phosphon, phosphon D ; tributyl [2,4 - dichlorobenzyl] phosphonium chloride) (Figure 2.9) was released in 1957 by Mobil Chemical. Phosphonium compounds had been recognised inhibitors for several years prior to this release (Anonymous 1955). The compound is another growth retardant affecting internode length which inhibits the enzymes involved in kaurene synthesis rather than the kaurene oxidation sequence of reactions (Davies 1995). This was shown by Kende *et al.* (1963) from mycelial and dwarf pea bioassays. Applied as a

soil drench the compound is bound strongly in soil and can only be removed by roots (Cathey 1975). It has been used to control pot grown chrysanthemum but foliar sprays were toxic to plants causing chlorophyll destruction (Cathey 1975). In roses it increased production of lateral shoots leading to more flowers but these were smaller than normal (Cathey 1975). Cathey (1961) found histological changes in stems of *Lilium longifolium* Thunb. where the layer of sclerenchymatous cells adjacent to the cortex was absent. This reduced robustness of the stem which was no longer able to support the inflorescence.

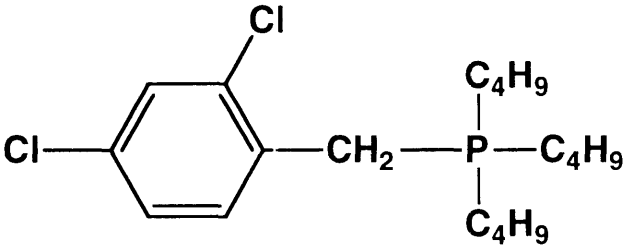


Figure 2.9 Chemical structure of chlorphonium chloride (phosphon)

Phosphon inhibits growth of Arizona cypress and coulter pine (Pharis *et al.* 1967) but promoted growth in sycamore (Cathey and Stuart 1961).

From the current literature the effectiveness of chlorphonium chloride as a growth retardant is very variable.

2.1.7 Dikegulac

Sodium dikegulac (syn. Atrinal®, dikegulac ; sodium 2,3 : 4,6 - di - O - isopropylidene - 2 - keto - L - glutonate) (Figure 2.10) was described by Bocion and de Silva (1977) and released by R. Maag Ltd. based in Switzerland. The compound is an intermediary in the synthesis of L-ascorbic acid and had successfully inhibited growth in several grasses (*Hordeum vulgare* L., *Lolium perenne* L. and *Digitaria sanguinalis* (L.) Scop.). The compound inhibited DNA synthesis in the apical meristem and is believed to be transported via the phloem (Group VII). GA_3 counteracts the growth retarding effect of dikegulac suggesting the compound behaves in a similar manner to chlormequat and other quaternary ammonium compounds (Bocion and de Silva 1977). Dikegulac reduces apical dominance and encouraging lateral shoots to form. As a result more flowers are usually produced. Parthenocarpy was also shown to develop in treated pear trees (Bocion and de Silva 1977).

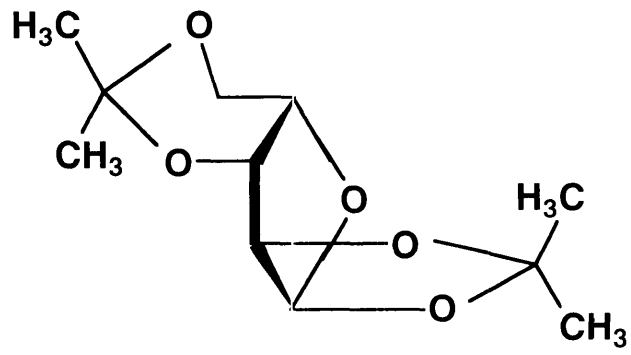


Figure 2.10 Chemical structure of dikegulac

Atrinal has been recorded as causing phytotoxic symptoms. *Eucalyptus* (Sachs *et al.* 1975b), sycamore and white pine (USDA 1976) were some species detrimentally affected. Application of this compound is problematic and phytotoxicity appears to be common amongst a number of tested species.

2.1.8 Mefluidide

The next growth regulator of consequence was **mefluidide** (5 - [trifluoromethanesulphoamido] acet - 2,4 - xylidide) introduced by the 3M Company mainly for the control of turf growth (Figure 2.11) and sold under the commercial names of Vistar® and Embark®. Considered for use as a tree growth regulator on red maple (*Acer rubrum* L.) and pin oak (*Quercus palustris* Munchhausen) (Sterrett 1980), but seems not to have been used much outside the area of grass control.

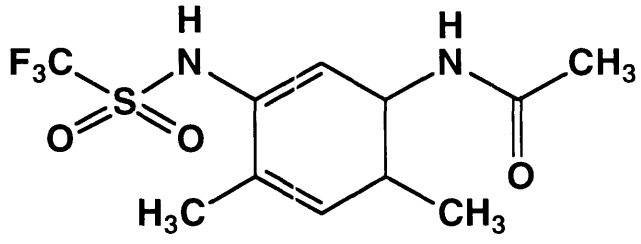


Figure 2.11 Chemical structure of mefluidide (Atrinal)

Several regulators shortly followed mefluidide, but none have made an impact on horticultural, agricultural or arboricultural fields of research.

2.1.9 Mepiquat

Mepiquat chloride (1,1 - dimethylpiperidinium chloride) (Figure 2.12) was introduced by BASF to reduce excessive vegetative growth in cotton (Heilman 1981). Belongs to the group of quaternary ammonium compounds which inhibit gibberellin

biosynthesis at the steps from GGPP (geranylgeranyl pyrophosphate) to *ent*-kaurene. Mepiquat-chloride injected into apple (*Malus domestica* Borkh.) seedlings had little or no effect on terminal extension growth (Miller 1982). Investigations have not progressed significantly beyond this point.

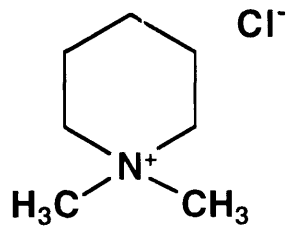


Figure 2.12 Chemical structure of mepiquat chloride

Following this compound a suite of new growth regulators were released based around a triazole ring. These triazole growth regulators are discussed more fully in the next section.

2.1.10 Other Plant Growth Regulators (Short Summary)

Apart from those detailed above there have been numerous introductions of novel PGRs since the 1960s. Most of these reached only their experimental stage and did not proceed to commercial or widespread application. Many display fungicidal properties and enantiomers or stereoisomers have been found to have plant growth regulating activity.

i) Ternary Sulphonium Carbamates

Developed in 1976 by The Boots Company these consisted of ternary sulphonium carbamates very soluble in water and alcohols but insoluble in non-polar solvents. Reduction in internode extension results in a dwarfed plant which was found useful in preventing lodging in soybeans.

ii) Phenyl Thiadiazole

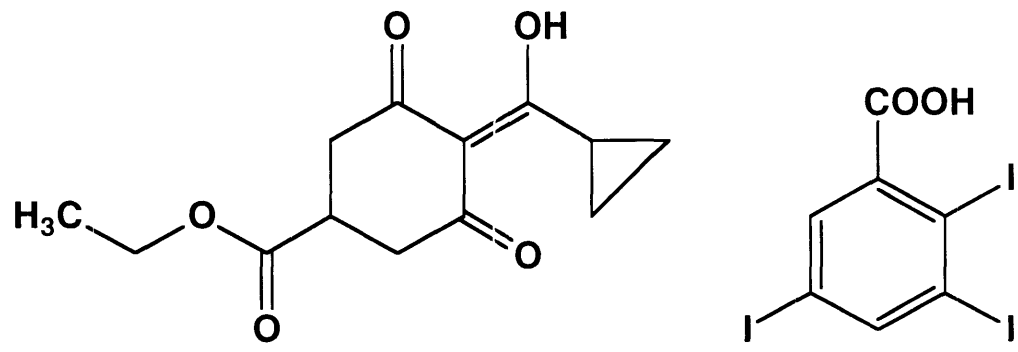
Synthesised by Fisons Agrochemical Division, UK in 1969. A phenyl thiadiazole [(3 - phenyl - 1,2,4 - thiadiazol - 5 - yl) thio] plant growth regulator with low toxicity which is believed to interfere with IAA transport and inhibiting apical dominance temporarily. Applied as foliar spray as it causes abnormal root growth as soil drench and needs a wetting agent to optimise application. Believed useful to promote branching in young fruit trees and chrysanthemum.

iii) Acylcyclohexanediones

Includes compounds such as cimectacarb (CGA-163935) (Figure 2.13.a) and prohexadione. Blocks the dioxygenases that catalyse the late steps of GA biosynthesis (Rademacher 1992). High levels of applied acylcyclehexanedione can cause an increase in anthocyanin production. Mainly used to control shoot length in grain crops such as wheat, rice, barley and canola.

iv) AMO-1618

AMO-1618 (ACPC) is a quaternary ammonium compound ([2 - isopropyl - 4 - (trimethylammonium chloride) - 5 - methylphenylpiperidine - 1 - carboxylate]) which blocks enzymes involved in kaurene synthesis (A activity). More specifically the cyclisation of GGPP to CPP (copalyl pyrophosphate). Many other inhibitors block the next step of oxidation of ent-kaurene to *ent*-kaurenol (B activity). Also inhibits synthesis of sterols and other triterpenes (Moore 1979). Selected by Wirwille and Mitchell (1950) it has been used to reduce shoot length in a range of tree species (Pharis *et al.* 1967) and *Chrysanthemum morifolium* (Sachs *et al.* 1960).



a) **b)**

Figure 2.13 Chemical structures of; **a)** cimectacarb and **b)** TIBA.

v) UNI P293 (UBI P293)

Introduced by Uniroyal Inc. (2,3 - dihydro - 5,6 - diphenyl - 1,4 - oxathiin) as a localised growth inhibitor. Inhibits cell division and elongation and promotes apical dominance by chemical disbudding in *Chrysanthemum morifolium*. Seems closely related in effect to dimethipin (DEF ; 2,3 - dihydro - 5,6, - dimethyl - 1,4 - dithiin - 1,1,4,4 - tetraoxide) used as a leaf abscission agent before harvesting of cotton. Shown to be phytotoxic to several tree species (Uffernan *et al.* 1979) and probably not widely used in that field.

vi) TIBA

2,3,5 - triiodobenzoic acid is an auxin transport inhibitor which reduces stem growth (Galston 1947, Zimmerman and Hitchcock 1942) (Figure 2.13.b). Luckwill (1981)

classes it in Group (III) as it blocks movement of auxin to the terminal bud. Used on soybeans it reduced plant height and petiole length while stimulating branching and fruit set. Not successful as a tree growth regulator where it caused phytotoxic symptoms in American elm (*Ulmus americana* L.) (USDA 1974, 1975).

vii) XE-1019 (possible synonym S-3307)

An enigmatic compound used as a tree growth regulator (TGR). Subsequently became known as uniconazole (Figure 2.14) and marketed under various names including Sumagic® and Prunit®. Very closely related to the fungicide tebuconazole (1 - (4 - chlorophenyl) 4,4 - dimethyl - 3 - 1 (H - 1,2,4, - triazole - 1 - ylmethyl) pentan - 3 - ol)) released by Bayer in 1981. This is not surprising as different enantiomers or stereoisomers of triazole growth retardants often show fungicidal activity.

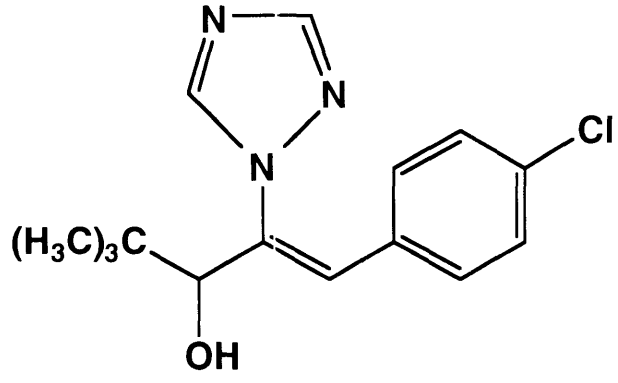


Figure 2.14 Chemical structure of uniconazole

viii) DCPTA

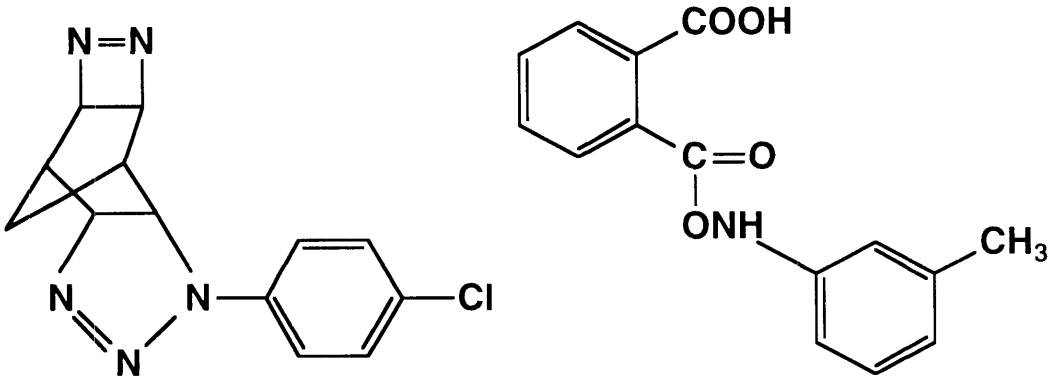
A tertiary amine bioregulator with potential for increasing crop productivity at high light intensities. Reduced number of days from planting to maturity of *Narcissus* sp. L., *Tulipa* sp. L. and *Hyacinthus* sp. L. (Meerow *et al.* 1994).

ix) Tetcyclacis

Coded as BAS 106..W and manufactured by BASF, tetcyclacis (5 - (4 - Chlorophenyl) - 3,4,5,9,10 - pentaaza - tetracyclo - 5.4,$10^{2,6}$,$O^{8,11}$ - dodeca - 3,9 - diene) (Figure 2.15.a) is a norbornenodiazetine derivative with a triazole component. Tetcyclacis inhibits both cell division and longitudinal cell growth in plants. The compound inhibits gibberellin biosynthesis at the same places as paclobutrazol (Rademacher *et al.* 1984).

x) MTPA (N - *m* - tolylpthalamic acid)

One of a series of active arylpthalamic acids (e.g. Duraset®, Tomaset®) (Figure 2.15.b) which blocks auxin and gibberellin transport in tomato plants causing a proliferation of lateral shoots which are reproductive (Luckwill 1981).



(a) **(b)**

Figure 2.15 Chemical structures of **a)** tetcyclacis and **b)** MTPA.

xi) Miscellaneous fungicides used as PGRs

Triadimefon sold under the name Bayleton® and triadimenol (Figure 2.16) both exhibit plant growth regulating activity. Both compound affect sterol biosynthesis in some fungi (Henry 1985).

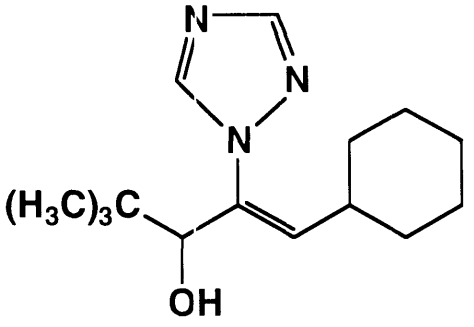


Figure 2.16 Chemical structure of triadimenol

3. Paclobutrazol

3.1 Discovery

Paclobutrazol (PAC) was discovered in the 1976 (Roberts and Hooley 1988) and the US patent for the product was filed as early as 1977.

In 1981, Balasubramanyan and Shephard patented (U.S. Patent 4,243,405) groups of imidazole and triazole compounds which had shown fungicidal and plant growth regulating activity. The patent states that

'the plant growth regulating effects of the compounds are manifested as for example a stunting or dwarfing effect on the vegetative growth of herbaceous mono- and di-cotyledonous plants'.

The compound was introduced by its code number, PP-333, by Lever *et al.* (1982) at the British Crop Protection Conference. Lever *et al.* (1982) described PP-333 as *'a new broad spectrum growth retardant with a wide range of potential uses'*. In their brief discussion they state the compound *'opens the way to totally novel opportunities for plant growth regulation which will be selectively developed'*.

Certainly, PAC has become a widely utilised plant growth regulator. New uses have been discovered and it is effective on a wide range of plants.

3.2 Chemical characteristics

Paclobutrazol (2*RS*, 3*RS*) - 1 - (- 4 - chlorophenyl) - 4,4 - dimethyl - 2 - (1H - 1,2,4 triazol - 1 - yl) pentan - 3 - ol) (Figure 17) is a triazole plant growth regulator developed released by ICI U.K.

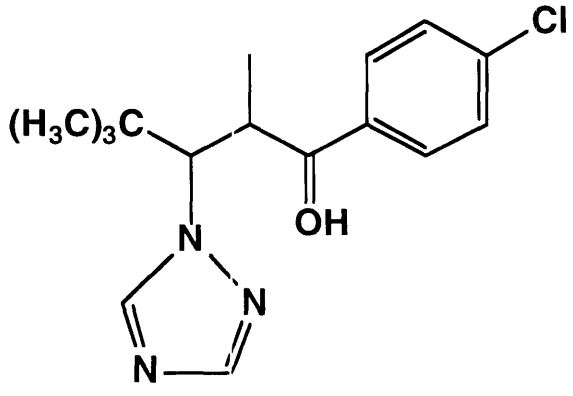


Figure 2.17 Chemical structure of paclobutrazol

3.3 Physical characteristics

The characteristics of PAC have been intensively studied. The compound has a molecular weight of 293.5 and in its native state is a white, crystalline solid. Supersaturated solutions of the commercial product, Clipper® have yielded large

crystals in the hexagonal system (Witchard unpubl.). Melting point is 165.6 °C and it has a density of 1.22 g / cm^3.

Solubility of the compound is variable with it being sparingly soluble in water (35 mg / L) but extremely soluble in other non-polar solvents such as methanol (15%) and ethanol (7.5 %). The compound becomes less soluble in polar solvents (xylene 6%, hexane 1%). The solubility in primary alcohols and not in water is an interesting feature.

Clipper®, contains 20 g / L of active ingredient (PAC) in 77.6 % ethanol. Paclobutrazol can be dissolved to a concentration of 75 g / L in pure ethanol.

Toxicology of the product has also been tested. PAC has low mammalian toxicity. Acute dermal toxicity LD_{50} of PAC is greater than 1,000 mg / kg body weight. A 70 kg human would need to absorb 70 g of Clipper® to display signs of dermal toxicity. Other organisms tested include Mallard duck (acute oral LD_{50} > 7,900 mg / kg). Rainbow Trout (96 h LC_{50} 27.8 mg / L) and bee (acute oral 'no effect' > 2 µg / bee).

3.4 Biochemical characteristics

Transport and mobility of PAC is primarily via the xylem (Quinlan and Richardson 1986, Intrieri *et al.* 1987, Early and Martin 1988, Hunter and Proctor 1990) but there is evidence to suggest that in some species it may also be transported through the phloem (Witchard, unpubl.). PAC inhibits the production of gibberellic acid.

The GA cycle begins with the conversion of acetyl CoA to mevalonic acid (Figure 2.18). After a number of pyrophosphate steps kaurene is produced proceeding to the alcohol, aldehyde and acid phases and on to producing GA itself. Some commercial growth retardants such as Phosphon D and CCC or Cyocel only attack the link between GGP and CPP. Ancymidol, affects the step from kaurene to kaurenoic acid. PAC, by comparison, can break the cycle at three points anywhere between the steps from kaurene to kaurenoic acid. Exactly how this is done is conjectural but it would appear that cytochrome p_{450} must unite with a protein to catalyse these three steps in the cycle. PAC binds to the bonding site between the protein and cytochrome, preventing the union required in the production of GA (Anonymous 1982).

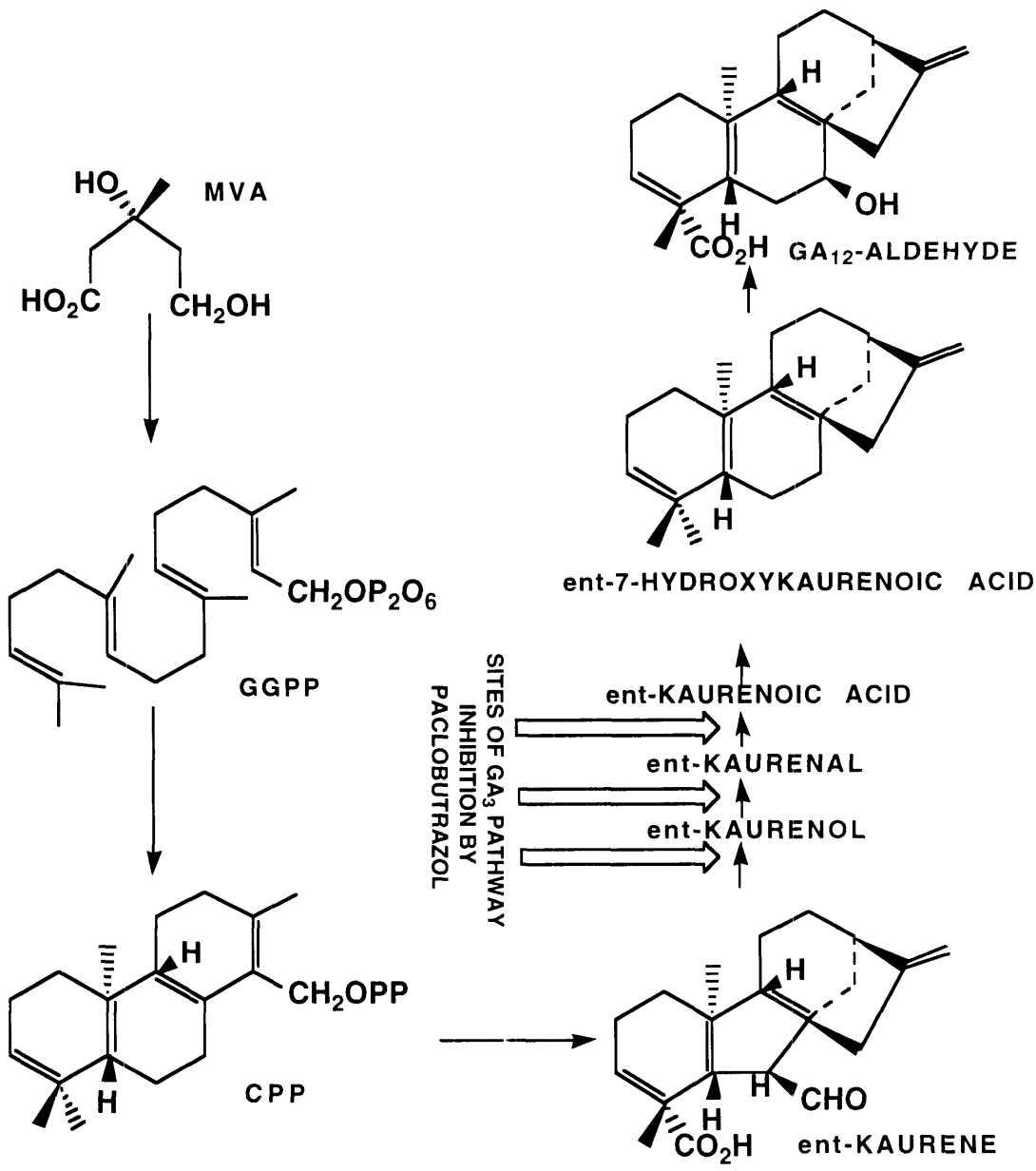


Figure 2.18. Overview of the GA biosynthesis pathway and the sites of inhibition by PAC (adapted from Salisbury and Ross 1985).

4. Effects of Paclobutrazol on plants

4.1 General Introduction

The main effect of PAC is in the inhibition of shoot growth and extension. This effect has been well documented and is the original basis for the use of PAC as a growth regulator. However, depending on the enantiomer present, PAC may also display fungicidal properties. PAC contains two chiral centres which allows the possibility for two pairs of enantiomers ; RS, SR and RR, SS (Lenton 1987). Because each enantiomer is not mirror image, they are termed diastereoisomers. The 2R,3R enantiomer is fungicidal in PAC while the 2S,3S enantiomer has the plant growth regulating properties (Figure 2.19). Most growth regulators will contain a mixture of these isomers.

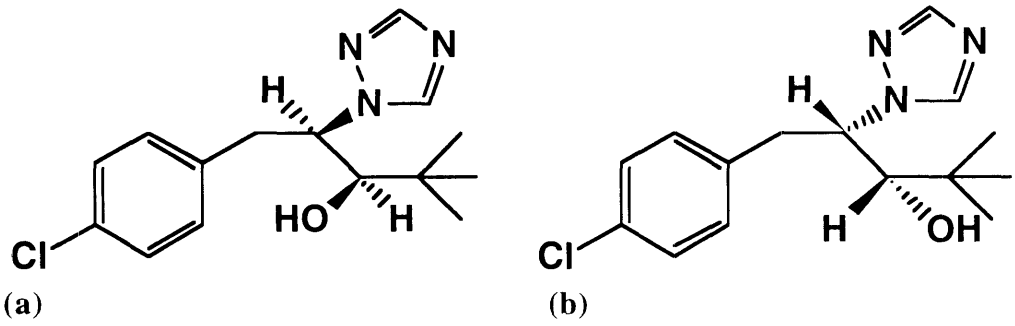


Figure 2.19 Enantiomers of PAC ; **a)** 2R, 3R and **b)** 2S, 3S forms.

Once the credentials of PAC as a growth inhibitor had been established in the early 1980s researchers mobilised to take advantage of its effects on their particular plant of interest. Unfortunately, the quality of the research varied considerably. Not only were established research bodies conducting controlled experiments (e.g. Long Ashton, ICI Plant Protection Division) but a number of unregulated and *ad hoc* trials were conducted by electrical utilities in the U.S. (e.g. Florida Light and Power). The value of such work might be easily underestimated however, their contribution to our current knowledge of PAC influence on a wide broad suite of species has been useful. By comparison, scientific institutions and government funded research organisations have tended to focus closely on individual species of interest such as apples, pears, wheat and other commercial crops.

The most common experimental plant species treated with PAC is apple (*Malus domestica* Borkh.) but this was not the first species to be tested with PAC (see England 1978). The range of plants tested with PAC covers trees (conifers and flowering plants), shrubs, herbs and grasses. Within these groups broad categories of interest

can be identified including ornamental p ants, crop plants, horticultural plants and street trees.

Standard growth trials with many species expanded in the 1980s. Experiments with PAC tested potted plants, plants grown from cuttings and plants *in situ*.

Some of the earliest trials using PAC (initially known by its code name of PP333) were conducted by England (1978) with amenity grasses. That same year, Hebblethwaite *et al.* (1978) experimented with grass seed crops using PP333 as a method of reducing lodging and thus, increasing yield. Many papers dealing with PAC tend to group the prevention of a physical constraint with an improvement in yield. In most cases it is best qualified that removal of a constraint on a reproductive plant by PAC does not *a priori* mean improved yield. Certainly, in the study by Hebblethwaite *et al.* (1978) plants not treated with PAC would fall over and therefore not have as great a yield as those plants allowed to stand, but this will not always apply. In the same year, Cohen (1978) published what is possibly the first international paper investigating the effect of PAC on the growth of three ornamental species. Within this single year PAC had been applied to grasses (amenity and cereal crops) and ornamental plants.

Two years later, Shearing and Batch (1979) were testing more amenity grasses with PAC and hailing the potential opportunities for the compound as a management tool of amenity turf such as perennial ryegrass (*Lolium perenne* L.). During this period great advances were also made in elucidation of the GA biosynthesis pathway by workers such as Graebe *et al.* (1980) and Stoddart (1980). It seems ironic that while scientists were experimenting with PP333 without knowing its exact mode of action, the intricacies of the GA pathway, which PAC inhibits, were being discovered.

By 1981, the use of growth regulators in general crop production was becoming so widespread that a book was published to encompass 'a new and rapidly developing technology concerned with the control of crop growth by specific growth regulating substances' (Luckwill 1981). Quite astonishingly, there was no mention in this book of PAC. The reason for this may have been influenced by its restricted usage and control by ICI. Indeed, at this stage, PAC was still known as PP333 but was acquiring a reputation as a 'hot' commodity amongst some researchers. And, to the research advantage of PAC, there were still no other triazole growth retardants available on the market the year this book was published. In the same year, Hardin and Stutte (1981) published a method for detection of plant hormones using HPLC, and from this small input a new (albeit future) scientific field opened up for the research and detection of PAC in plants.

Finally, in the same year as these publications, came the debut of PAC as US Patent No. 4,243,405, granted to Balasubramanyan and Shephard (1981) in August. Although it had been filed in 1977, the patent approval had only just been granted. The patent covered all triazoles of the formula shown in Figure 2.20. Until this point the compound had been known by its code name of PP333, and afterwards as paclobutrazol.

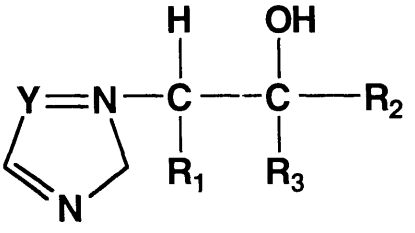


Figure 2.20 The triazole formulae patented by Balasubramanyan & Shepherd (1981). R_1 is alkenyl, alkynyl or arakyl; Y is =N-, R_2 is cycloalkyl, alkyl or haloalkyl; R_3 is H, methyl or alkenyl, or esters, ethers, salts and metal complexes thereof.

Renewed investigation into the mode of action and effects of this new plant growth regulator began. In 1982, Barrett and Bartuska (1982) conducted an experiment which found that the stem elongation effects of PP333 were dependent on the site of application. Container grown plants of bean (*Phaseolus vulgaris* L.) and chrysanthemum (*Chrysanthemum morifolium* Ramat) were treated with PP333 through their roots, or shoot or leaves. The least stem elongation occurred when PP333 was applied to the shoot rather than the roots or the leaves.

From this point in time the list of potentially useful effects elicited in plants by PAC become extensive, and this review can only attempt to present a segment of this work. No doubt there are many experiments and observations which go undocumented, or are not tested further.

The following review is divided into sections encompassing the effects on the following plant parts;

a) stems,
b) leaves,
c) roots,
d) flowering, and,
e) seed/fruit set.

Since the prime target of PAC is the reduction of shoot growth in plants it seems a logical position from which to begin this review. The suggested mechanism of this effect has already been presented. The range of plants tested with PAC to reduce shoot growth is extensive but in recent years, the number of papers examining this process has declined, perhaps as more novel growth regulators have been developed. Another reason for the reduction in papers dealing with PAC may be that there are many plant species that have been found to be unaffected by this compound. Aside from variability in the environment of the plants being tested, there is something more intrinsic which allows particular plants to resist the regulating effects of this compound. Speculation on mechanisms as to why PAC is not always effective are presented in a later chapter.

4.2 Effects on shoots and stems

The number of papers written in this area of PAC responses is extensive. When plants respond to applications of PAC the response generally falls into three areas; inhibition is dependent on the concentration applied or, inhibition is independent of the concentration applied.

4.2.1 Inihibition dependent on concentration applied

Keever *et al.* (1990) used foliar sprays or soil drenches of PAC on eight woody landscape plants (*Euonymus japonica* H. Jaeg. cv. Microphylla, *Ilex cornuta* Lindl. & Paxt. cv. Burfordii nana, *Ilex crenata* Thunb. cv. Compacta, *Juniperus coniferta* Parl. cv. Blue Pacific, *Rhododendron obtusum* Planch cv. Hino Crimson, *Rhododendron indicum* L. cv. Formosa, *Photinia* x *fraseri* Dress and *Ligustrum japonicum* Thunb. cv. Aureo-marginatum). As the concentration of PAC was increased (from 6 - 100 mg PAC drench / pot or, 250 to 2000 ppm spray), there was a correlative decrease in stem length. Sturt's Desert Pea (*Swainsona formosa* (G. Don) J. Thompson) treated with increasing concentrations of PAC (0.1 - 50.0 mg a.i. / plant) also showed a correlative decrease in plant height (Hamid 1996).

4.2.2 Inhibition independent of concentration applied

Evidence that increasing PAC concentration eventually has little effect on stem growth beyond a certain level is less easily found. In an experiment by Cox (1990), the most effective application rate of PAC to street trees of *Lophostemon conferta* ((R.Br. P.G. Wilson & Waterhouse) was 70 ml / hole. At 100 ml / hole the actual reduction in shoot growth was less. One might argue that this is not actually a change due to concentration but of volume. That in fact, Cox (1990) was not changing the

concentration of PAC but merely the amount introduced into the plant. Nevertheless it does indicate that applying more PAC to produce more shoot inhibition is not effective for all species. Such a limit is applicable to many PGRs and hormones.

4.2.3 Lack of inhibition

The third response to consider is that PAC has no effect or, at worst, a negative effect on the subject. There is anecdotal evidence that PAC is relatively ineffective on ashes (*Fraxinus* spp.), London plane trees (*Platanus* x *hispanica* cv. Acerifolia), many conifers and, some fast growing tree species (e.g. *Jacaranda mimosaefolia*, *Flindersia* spp.) . The significance of this lack of response and its possible causes will be discussed more fully later in this thesis.

There is scientific documentation and not just anecdotal evidence to support the claim that PAC is not always a good choice for stem inhibition in plants. In the case of stems of lowbush blueberry (*Vaccinium angustifolim* Ait.) foliar sprays of PAC during their sprouting year had no effect on stem growth compared with untreated plants (Lewis and Ju 1993). To confirm that this response was not isolated to a sprouting year application, stems were also sprayed in their fruiting year. The result was the same. In another experiment the growth response of begonia (*Begonia semperflorens* hybrid complex), vinca (*Catharanthus roseus* ((L.) G. Don f.)), impatiens (*Impatiens sultani*) and zinnia (*Zinnia elegans* Jacq.) to foliar sprays of PAC was examined. Of these four species, zinnia was found to be unaffected by the PAC treatment. Nor was zinnia affected by uniconazole (XE-1019), another triazole growth retardant. This suggests that PAC effects are species specific.

Such a response would suggest that plants which are not affected by PAC are unlikely to be affected by any triazole growth regulator. However, in a rice bioassay study, Takehashi *et al.* (1986) found that uniconazole was 100 times more effective than PAC at equivalent molecular concentrations. This was confirmed in a study with tree peony (*Paeonia suffruticosa* Andr. cvs. Taiyoh & Hanakisoi) where Hamada *et al.* (1990) found that uniconazole was also more effective than PAC.

4.2.4 Persistence of PAC

Another feature of PAC is its persistence in soil environments. The desirability of such persistence is dependent on the required outcome. For example, if growing ornamental plants for a commercial market, persistence of PAC from season to season may be undesirable if you wish to grow a tall stemmed variety the following

season. Persistence would also be undesirable in a short rotation cropping system. On the other hand, persistence of PAC in maintaining a dwarf habit in conifers for commercial sale might be advantageous. Just how persistent is PAC?

In small scale aquatic systems, the $T_{1/2}$ of PAC was 24.4 d compared to 5.2 d for uniconazole (Chand and Lembi 1994). However, in the soil it was found that PAC persisted for the duration of the 168 d sampling period whereas uniconazole had dissipated after 102 d. In another study, Karageorgiev and Marovdiev (1989) calculated that the half-life of PAC in apple (*Malus domestica* Borkh.) orchards following triple spraying was 10.9 - 11.9 d. According to very recent work by Jackson *et al.* (1996), the half-life of PAC in soil at 25 °C is 4.5 y while Hillier *et al.* (1992) claim the $T_{1/2}$ is 3 - 12 months depending on temperature and organic matter. This is a very long period of time and yet, with the many experiments to date only a few have indicated a carryover effect from season to season or trial to trial. And even these are not consistent in the time that PAC persists. We could conclude from this evidence that persistence is less likely in a small scale system (in pots, small ponds or glasshouses) than in the field. But again, there are exceptions.

An example of useful persistence can be cited from Deans (1989) who found that pot grown radiata pine (*Pinus radiata* D. Don) had shoot growth inhibited by PAC for approximately 8 months. And in the case of pot grown white cedar (*Melia azedarach* L.) control lasted for 3.5 y.

Persistence viewed in a positive role leads to one of the most useful applications for PAC; the control of shoot growth in street trees under powerlines. In this situation the longer the persistence, the more cost effective is the method of control. The advantages and disadvantages of implementing a chemical growth control system is discussed elsewhere in this thesis.

Deans (1989) conducted a series of field trials to determine the effectiveness (which includes persistence) of PAC in inhibiting the growth of trees under powerlines. He found that he could obtain good control in *Melia azederach* L. of between 42 % and 62 % reduction in regrowth compared to controls. This extended the time between tree trimming periods from 12 - 18 months to 36 months. *E. camaldulensis* (Dehnh.), *Eucalyptus odorata* Behr and *E. spathulata* Hook. were also controlled by 40 %, 80 % and 70 % respectively over untreated trees.

The ability of PAC to control growth in street trees, particularly those under powerlines, has implications in time and cost savings for those organisations entrusted

with the control and management of such trees. The potential for extending the period of time between tree trimming cycles is the most tangible benefit.

To this point, PAC has been discussed in terms of its main effect, that of shoot growth reduction. In terms of ancillary effects on stems, several responses have been documented.

4.2.5 Changes in nutrient status

PAC has been documented as affecting the nutrient status of plant stems and shoots. We could predict that any compound which inhibits shoot growth would result in *de facto* partitioning of resources within the plant, thus influencing nutrient status. The levels of N, Ca, Mg, Zn and Cu in apple (*Malus domestica* Borkh.) seedling stems increased in response to treatment with soil drench PAC (Swietlik and Miller 1985). There was also a noted increase in levels of manganese within plants. While increases in macronutrient levels might be considered mainly beneficial during plant growth, a rise in the level of Mn may be beneficial or deleterious to the plant. Swietlik and Miller (1985) also noted that the level of K decreased with increasing levels of PAC. More comprehensive studies into nutrient status have been conducted with plant leaves, and are discussed in a later section.

4.2.6 Changes in carbohydrate levels

Beside nutrient levels there have been studies documenting changes in the levels of particular carbohydrates within PAC treated plants. Soluble carbohydrates (sorbitol, glucose, fructose, sucrose) levels in PAC treated apple (*Malus domestica* Borkh..) wood was generally higher than in control plants (Wang *et al.* 1986a). As PAC caused a decrease in vegetative growth, resources were partitioned into both new and previous year's wood. The changes observed in plants are not biochemical responses, but a result of partitioning of materials due to PAC inhibition of shoot growth. While this is not a novel response, the effects can be used to manipulate growth, flowering and fruiting in plants. And this brings us to another response resulting from the experimentation with PAC.

4.2.7 Reduced chilling injury

Relating also to carbohydrates, Raese *et al.* (1978) found that high levels of sorbitol and/or sucrose, coincided with increased levels of chilling resistance in many plants, as caused also by low levels of starch (Siminovitch *et al.* 1953). This accounts for the

observations of Holubowicz (1983) who found that acceleration of acclimatisation to cold conditions was enhanced in two year old potted peach (*Prunus persica* L. cv. Rakonievicka) seedlings treated with a foliar spray of PAC. After two applications of PAC, plants survived temperatures to - 18 °C compared to only - 12 °C in control plants. Endogenous ABA is also believed to prevent chilling injury in some plants and increase freezing tolerance in others. For example, plants of *Actinidia arguta* (Siebold & Zucc.) treated with PAC had increased levels of endogenous ABA and lower electrolyte leakage than control plants suggesting that PAC may be effective in increasing cold hardiness in this species (Tafazoli and Beyl 1993).

4.2.8 Reduced heat / moisture stress

Not only has PAC been documented as reducing chilling and freezing injury, it can, in some cases, also reduce effects of heat or moisture stress. Heat stress in wheat (*Triticum aestivum* L.) seedlings results in generation of active oxygen. Treatment of seedlings with PAC increased the activities of catalase and peroxidase and protected plants from free radical damage through increased anti-oxidant activity (Kraus *et al.* 1995). Unlike changes in nutrient or carbohydrate status, this effect is independent of partitioning, and indicates an entirely new area of research.

4.2.9 Summary

Perhaps the biggest challenge facing the use of PAC is our knowledge that it is not universally effective and, though PAC was never billed as a definitive product, it has proven to be the most promising growth inhibitor synthesised to date. But even in situations where it is effective its inhibition varies from species to species. There is already a reasonable database of knowledge to inform users of the expected effectiveness of the compound in their particular situation but more species need to be tested in specific situations to fully evaluate its usefulness. However, the prospects for further research with PAC may diminish as newer plant growth regulators are developed. A second difficulty with PAC is its persistence. While reports seem to vary, PAC does appear to persist for extensive periods of time from months to years. Persistence may be viewed as a positive or a negative effect as discussed previously in this section. The reduction of persistence may only require more careful procedures during application. For example, foliar spray and soil drench techniques invariably leave residue on surfaces which may persist into subsequent experiments. The technique of trunk or stem injection holds more promise in reducing the possibility of persistence as it minimises dispersal of the compound by being introduced directly into the transport system of the plant. I can envisage that further experimentation with PAC

will shift from shoot inhibition to less explored fields of research such as minimising chilling injury or moisture stress, helping manipulate plants which are adaptable to a wider range of environments.

4.3 Effects on leaves

Research into the effects of PAC on leaves began shortly after investigation of stem effects. One typical observation was that many species acquired darker green leaves after treatment. The range of plants in which this was observed included lowbush blueberry (*Vaccinium angustifolium* Ait. (Lewis and Ju 1993), Japanese honeysuckle (*Lonicera japonica* Thunb. var. *aureoreticulata* (Kwack and Hwa 1990) and *Liquidambar styraciflua* L. (this thesis). Most researchers have accepted this phenomenon without further inquiry because, in many cases, it enhanced the appearance of ornamental plants or had little bearing on the outcome of their research in other areas. The colour change, having now been investigated, is based around two hypotheses; that more chlorophyll was being produced or, that less photosynthetic tissue was being produced. Here we have a dichotomy between a physiological response and an anatomical response.

4.3.1 Physiological changes causing darkening

One of the earliest investigations of this type involved apple seedlings (*Malus domestica* Borkh. cv. York Imperial) which had been treated with nutrient solution + PAC. These seedlings produced leaves in which relative chlorophyll content on a leaf area basis was increased (Steffens and Wang 1984). Here was direct evidence to link PAC with an actual increase of chlorophyll content in leaves. In another experiment, application of soil drench PAC also increased chlorophyll content of leaf discs from cucumber (*Cucumis sativus* L.) and zucchini (*Cucurbita pepo* L.) (Wang 1985). However, not all species appear to respond in the same manner.

In four native Australian plants (*Chamaelaucium uncinatum* Schaeur, *Correa alba* Andrews, *Crowea exalata* F. Muell. and *Dampiera trigona* R. Br.) treated with 5.0 mg PAC / plant, no difference was found in the levels of chlorophyll a or chlorophyll b compared to untreated plants (Stewart 1991). Both *Correa* and *Crowea* are members of the Family Rutaceae which are known to react unusually to applications of PAC (see Bausher and Yelenosky 1987). Henderson (1993) found that *in vitro* plants of *Anigozanthos bicolor* Endl. grown under high humidity had slightly reduced amounts of chlorophyll compared to control plants. Just as there are plants whose stem growth is unaffected by PAC there are plants whose leaves respond differently.

Another example is apple (*Malus domestica* Borkh.) trees treated with foliar spray PAC whose leaves actually had lower chlorophyll contents than the control plants (Bonomo *et al.* 1989).

Where PAC does cause a response in leaves it may not only be chlorophyll levels which are affected. Plants of variegated Japanese honeysuckle (*Lonicera japonica* Thunb. var. *aureoreticulata*) had PAC applied as soil drench or foliar spray. Leaves produced subsequent to treatment were darker green with elevated levels of chlorophyll and carbohydrate content compared to control plants (Kwack and Hwa 1990). Carotenoid levels were also measured but were found to be unaffected by PAC. By contrast, leaves of treated plants of lowbush blueberry (*Vaccinium angustifolium* Ait.) turned orange-red in fall after treatment with PAC while control plant leaves remained green (Lewis and Ju 1993). The orange-red colour is indicative of carotene pigmentation and its derivatives, and suggests that PAC had either accelerated the destruction of chlorophyll or enhanced the production of carotenoids, or both. In either case PAC is achieving an effect opposite to the usual darkening of green leaves.

4.3.2 Anatomical changes causing darkening

So far I have concentrated on the physical increase or decrease in chlorophyll production following application of PAC. The alternative idea is to consider that alterations in leaf anatomy and/or morphology, enhances the visibility of chlorophyll rather than there being an actual increase in chlorophyll production. An indication of this process is presented by Rao and Mendham (1991) who recorded that after treatment with soil drench PAC, leaves of chinoli (*Brassica campestris subsp. oleifera* x *subsp. chinensis*) and oilseed rape (*B. napus* L.), were thicker and darker green than control plants. Their work suggests that anatomical changes independent of chlorophyll increases can contribute to the process of darkening in leaves affected by PAC.

A reduction in the size of a leaf with maintenance of chlorophyll levels will inevitably produce darker green leaves because the concentration of chlorophyll has been elevated relative to the unit area of the leaf. PAC has the ability to reduce leaf size and area. Average leaf area of apple (*Malus domestica* Borkh.) trees treated with PAC was 20 % lower than for controls (Bonomo *et al.* 1989). In addition, overall leaf area (average leaf area x shoot number x node number) was reduced by 50 % in plants treated with soil drench PAC and 33 % lower in those treated with a foliar spray. In a different study, leaf area and dry weight was also reduced in plants of feijoa (*Feijoa sellowiana* O. Berg) and privet (*Ligustrum japonicum* Thunb.) treated with PAC (Martin *et al.*

1994). Smaller, thicker leaves were also observed and measured in seedlings of black locust (*Robinia psuedoacacia* L.) whose seeds had been pretreated with PAC solution prior to planting (Hui-Juan and Bin 1993).

The issue of leaf size and leaf weight has been touched upon in previous references. PAC has an effect on these parameters and this is indicative of the broad spectrum of retardation elicited by this compound.

4.3.3 Changes in nutrient status

As with stems, the nutrient status in leaves may be altered by PAC. Some representative examples of work are presented here.

Apple plants (*Malus domestica* Borkh.) have been used extensively for a range of PAC experimentation including determination of nutrient levels in leaves of treated plants. Seedlings treated with soil drenches of PAC produced leaves with increased levels of Ca and Mn, and reduced levels of K compared to control plants (Swietlik and Miller 1985). This evidence is similar to the nutrient status of stems reported by the same author and supports work by Bonomo *et al.* (1989) who found increased Ca levels and reduced K levels in leaves. This is supported in work by Atkinson and Crisp (1983) who found that potassium levels in apple leaves also decreased, while N and P increased. Finally, in another study which ignored potassium levels, the concentration of potentially toxic aluminium was reported to have increased in apple leaves relative to control plants (Numbere *et al.* 1992).

PAC also affects the tolerance of leaves to chilling injury, heat / moisture stress and, even SO_2 damage as claimed by Lee *et al.* (1985). In the experiment of Lee *et al.* (1985), snapbean plants (*Phaseolus vulgaris* cv. Blue Lake) were treated with soil drench PAC. A dosage of 0.02 to 0.5 mg / pot proved to be an effective protectant against SO_2 injury, chilling and heat stress compared to untreated plants. While there is no other evidence that PAC protects from SO_2 injury there is a great deal of evidence supporting its role in chilling injury and/or heat stress protection.

4.3.4 Reduced chilling injury

Increased chilling tolerance caused by PAC is the final issue in this section. As with stems, it is likely that the level of soluble carbohydrates present in leaf tissue is an indicator of its ability to tolerate freezing or chilling. One marker of this is leakage of electrolytes from leaf tissue which is believed to be indicative of the level of injury

sustained by a plant from freezing or chilling. In one experiment, the electrolyte content of gloxinia (*Sinningia speciosa* (Ker-Gawler Hiern)) leaves under chilling stress was inversely proportional to the dosage of PAC applied i.e. electrolyte levels were lower in PAC treated plants (Borochov and Shahar 1989).

Suitable plants to test the effect of chilling injury are found in the family Cucurbitaceae which are notoriously sensitive to chilling and are always destroyed by freezing events. The visual symptoms of chilling injury were delayed in PAC treated plants of cucumber (*Cucumis sativus* L.) and zucchini (*Cucurbita pepo* L.). Symptoms of chilling injury appeared after 4 - 5 d in PAC plants compared to 2 d in control plants with both groups subjected to a temperature of 5 °C (an injurious temperature for cucurbits) for the duration of the experiment (Wang 1985). One explanation behind this effect was suggested by Whitaker and Wang (1987). Working with cucumber plants they claimed that PAC had reduced the levels of leaf polar fatty acids at injurious temperatures and that the protection conferred by PAC appeared to increase with the duration of pre treatment. Whether this mechanism is specific to cucurbits or is more widely applicable is not known.

The above example is one of PAC minimising chilling injury. The extreme event is total freezing of the plant body. Even deciduous species at critically low temperatures may develop freezing injury. In the case of apple (*Malus domestica* Borkh. cv. Spur McIntosh/MM.106) the mean percent freezing injury caused by temperatures of - 25 °C and - 35 °C were significantly lower in PAC treated trees than control trees. Even greater protection was conferred through a combination spray of PAC + flurprimidol + thidiazuron (Coleman and Estabrooks 1993). Plants of winter rape (*Brassica napus* L.) treated with soil drench PAC had higher rates of recovery after being frozen at - 18 °C than control plants (Morrison and Andrews 1992). Again, whether this increased tolerance is due to physiological effects or merely anatomical alteration to the leaf is not known.

4.3.5 Reduced heat/moisture stress

The protection from heat stress may be associated with either a biochemical response, an anatomical response, or a mixture of the two. Black locust (*Robinia pseudoacacia* L.) seed was pretreated prior to planting by soaking them in a 250 ppm PAC solution. The resultant seedlings had smaller, thicker leaves and, under moisture stress, showed signs of only moderate wilting after 96 h. This compares favourably with untreated plants, which showed signs of wilting after only 12 h (Hui-Juan and Bin 1993). This part of the research is evidence for an alteration in leaf anatomy resulting in increased

drought tolerance. However, in the same study it was also found that levels of putrescine (a possible marker of cellular stress) was twice that in untreated seedlings compared to PAC plants after a period of prolonged moisture stress. Here is evidence of a biochemical alteration caused by PAC. Unfortunately we cannot be conclusive about this last statement as the level of putrescine might just as easily been caused by structural wilting of the leaves rather than the chemical interaction with PAC. In another experiment examining a chemical marker of stress, apple leaves (*Malus domestica* Borkh cv. York Imperial) of PAC treated plants evolved less ethylene than untreated plants when placed under moisture stress (Steffens and Wang 1984) which again suggests that PAC plants were not as stressed relative to the control plants having a higher tolerance to moisture stress. Again, there is no evidence in this report to indicate the thickness or size of leaves of untreated vs. treated plants. Whether PAC directly affected ethylene production or indirectly by reducing wilting is not clear.

Ancillary effects also known to be influenced or caused by PAC include delay of leaf flushing in cocoa (*Theobroma cacao* L.) (Omran and Fordham 1992), promoting growth of adventitious roots on leaves of rough lemon (*Citrus limon* ((L.) Burm. f.)) (Bausher and Yelenosky 1987) and increasing stomatal frequency in feijoa (*Feijoa sellowiana* O. Berg.) (Martin *et al.* 1994)

4.3.6 Summary

Unlike stem inhbition research using PAC, the effects of PAC on leaves are more amenable to future research. PAC not only inhibits leaf production but can significantly alter leaf anatomy. Applications of PAC might be used to reduce leaf size or number to enable plants to adapt to more extreme environmental conditions than in which they would normally grow. There are possibilities for extending fruit ripening seasons if the leaves are manipulated to survive the onset of light frosts. Less drought tolerant plants might be manipulated to survive in what would be otherwise be marginal environments. Another significant prospect is the promotion of adventitious roots on leaves. The range of species tested for this effect should be broadened to include mass produced plants such as roses or carnations with the potential for increasing production of these from explants stimulated into developing roots using PAC. Changing the levels of mineral nutrition in plants is another area of research. The manipulation of the nutrient status of plants with PAC could improve the partitioning of resources during fruit set and development. The only problem here is elegantly summarised by Bausher and Yelenosky (1987), ' Research (*into manipulation of mineral nutrient status)* can be summarised by saying that the levels of minerals throughout plants treated with PAC

vary from author to author and species to species. '. As with most areas of investigation involving PAC the results are often highly variable.

4.4 Effects on roots

While the effects of PAC on the growth of stems has been well documented, and on leaves adequately documented, for roots there is a relative paucity of information. In terms of transport *in vivo*, PAC has been found to move basipetally in *Ricinus communis* L. (Witchard, in press) and apple (*Malus domestica* Borkh.) (Early and Martin 1988). Whether these quantities are sufficient to elicit a response is not known. We can only surmise that the concentration of PAC must be at least similar to that of conventional growth regulators to be effective. Furthermore, what sort of response is expected? If we compare PAC to gibberellins then there is little actual effect to expect. However, this is not the case.

There is evidence that PAC can severely inhibit production of lateral roots in some species. The most exaggerated effects are documented in plants which are members of the citrus family (Family Rutaceae) which have been mentioned in an earlier section. When seed of 'Valencia' (*Citrus sinensis* (L.) Osbeck) oranges were soaked in PAC prior to planting the resultant seedlings had abnormally formed primary roots. And, when PAC was applied to untreated seedlings there was a significant inhibition of secondary root development compared to control plants (Bausher and Yelenosky 1987). In the same series of experiments, the seedlings treated with soil drench PAC also produced shorter and thicker roots with enlarged cortical cells.

4.4.1 Anatomical / Morphological changes

The developmental response of some root systems to treatment with PAC appears to have a common theme in the production of a swollen root apex. In an experiment with apple (*Malus domestica* Borkh.), soil drench PAC caused the roots to develop swollen apices similar to those found in citrus by Bausher and Yelenosky (1987). The cause of this response is speculative.

Plants of *Anigozanthos bicolor* Endl. grown *in vitro* and treated with PAC in the growing medium produced thickened rhizomes along with an absence of roots. Root length however, was unaffected (Henderson 1993).

Seeds of pea (*Pisum sativum* L. cv. Taichung No. 11) were treated with soil drench PAC after the roots had reached a length of 1 cm. Primary root extension was

subsequently inhibited and swelling occurred close to the apex resulting in shorter, thicker roots (Wang and Lin 1992). PAC was found to inhibit root elongation through increases in the final diameter of cells and suppression of their final length.

So, despite expectations, PAC has the ability to inhibit root growth and in this respect it cannot be considered an 'opposite' of GA (i.e. an anti-gibberellin) for, while it may directly inhibit GA production, its effects go beyond the bounds of GA developmental influence.

4.4.2 Production of adventitious roots

Another common response evident from investigation of root systems affected by PAC is the production of adventitious roots. Adventitious roots are those which arise in places on parts of the plant body they are not normally associated with. Many grasses produce adventitious roots, sometimes known as prop roots which form at or just above ground level. These roots provide extra stability to the plant. Already mentioned in a previous review section (leaves) was the production of adventitious roots on the leaves of rough lemon (*Citrus limon* (L.) Burm. f.) by Bausher and Yelenosky (1987). In that experiment roots were induced on petioles of leaves but were thick, and less likely to form secondary roots than comparable control plants. Adventitious roots have also been promoted on cuttings of *Plectranthus australis* R.Br. and *Phaseolus vulgaris* L. after soaking them in solutions of PAC for 24 to 40 h (Davis *et al.* 1985). Promotion of adventitious roots was also found in sunflower (*Helianthus annuus* L.) plants growing for 120 h in PAC + nutrient solution (Curry 1989). Production of adventitious roots in juvenile phase ivy was also promoted by applications of soil drench PAC (Horrell *et al.* 1990).

By contrast there are experiments in which PAC promoted rather than inhibited lateral root formation and other new roots. One example involved the application of soil drench PAC to pot grown, micropropagated apple (*Malus domestica* Borkh.) M.25 or Colt rootstocks. PAC increased root branching, diameter and production of new roots especially from the stem at ground level (Atkinson and Crisp 1983). This result merits closer examination. Root branching was increased, but root diameter was also increased. Adventitious roots also appeared. Both these features are typical of root responses to PAC. Only the production of increased lateral roots runs counter to the known effects of PAC. An increase in lateral root production has also been documented during the growth of Asian pears (*Pyrus pyrifolia* ((Burm. f.) Nakai). PAC applied as wettable powder to the soil promoted lateral growth of roots in the year following application of the compound (Huang *et al.* 1989). An 'increased' root

system was also documented in young apple trees by Numbere *et al.* (1992) after application of nutrient solution at concentrations as low as 0.10 and 0.15 ppm PAC. In another experiment, the fibrous root system of apple seedlings was increased by PAC applied to the nutrient solution (Steffens and Wang 1984). Many new short and enlarged roots were also produced during that experiment compared to the control plants. However, PAC had no effect on root length of cuttings of either *Plectranthus australis* R.Br. or *Phaseolus vulgaris* (L.) compared to cuttings soaked in water (Davis *et al.* 1985).

4.4.3 Changes in nutrient status

The levels of mineral nutrients within root systems affected by PAC have not been investigated as widely as the level of carbohydrates in root systems. The level of starch in fibrous roots of apple seedlings (*Malus domestica* Borkh.) was higher than in control plants (Steffens and Wang 1984). From this evidence it was suggested that this had resulted from inhibition of GA synthesis reducing the carbohydrates required by the shoots of the plant and allowing a build up of carbohydrates in the roots. The significance of this result may be illustrated by another experiment involving hydroponically grown sunflower plants (*Helianthus annuus* L.). These plants were grown in PAC + nutrient solution. At 24, 48 and 120 h plants were removed and the nutrient medium measured for carbohydrates. The level of fructose increased over the 120 h experimental period compared to controls. Levels of glucose were also significantly higher in the nutrient solution of treated plants (Curry 1989). Curry (1989) could not determine whether the effect was a result of PAC directly increasing membrane permeability (biochemical) or indirectly from redirected photosynthate partitioning (physiological). In either case he suggested that the increase in carbohydrate around the roots might lead to proliferation of microorganisms. This suggestion was not proven experimentally and considering lack of evidence linking PAC with proliferation of microorganisms the claim is somewhat spurious.

By contrast, Wang and Lin (1992) found that in pea roots (*Pisum sativum* L. cv. Taichung No. 11) that carbohydrate content was unaffected by PAC in seed treated with the compound.

In ancillary experiments, PAC has also been shown to reduce moisture stress in pea roots (Wang and Lin 1992) and increase anthocyanin production in carrot suspension cultures (Ilan and Dougall 1992) .

4.4.4 Effects on other vegetative propagules

Finally, the effects of PAC on root systems are not restricted to fibrous or tap root systems. Root tubers of gloxinia (*Sinningia speciosa* ((Ker-Gawler) Hiern.)) increased in size with PAC soil drench treatments while the water content was lower compared to controls (Borochov and Shahar 1989). This result might have been expected on the basis of the partitioning of resources resulting from PAC. Tubers would be a natural 'sink' for materials produced by the leaves especially where there was no active shoot growth occurring. An extension of this idea might be made in the field of potato production where increased tuber size and possibly production would be advantageous to the grower. PAC has also been shown to effectively reduce the production of propagules in purple nutsedge (*Cyperus rotundus* L.; estimated to be the 'world's worst weed' (Holm *et al.* 1977)) and completely eliminated production when applied as a soil drench at 2 and 8 mg PAC / litre of soil (Kawabata and de Frank 1993).

4.4.5 Summary

The effects of PAC on roots are more complex than we can ascertain. While there are degrees of commonality between some experiments, these effects are just as likely to be influenced by the type of developmental stage of the plant being tested (cutting, pot grown, seed or seedling) as the species itself. Certainly, the anatomical and morphological changes caused by PAC are of little value. Reduction in the growth and development of root systems severely inhibits the overall growth of the plant. Partitioning of resources resulting from PAC treatment may also increase exudation of carbohydrates around the roots which may be beneficial (e.g. proliferation of nutrient transferring microorganisms) or detrimental (e.g. proliferation of pathogenic microorganisms), to the plant. While the responses elicited by PAC on roots are novel they are otherwise scarcely beneficial and research would be better concentrated in other areas.

4.5 Effects on flowering

Flowering is a fundamental mechanism of plant development. PAC has been found to influence a broad range of characteristics associated with flowering.

4.5.1 Flower and inflorescence production

The most widely documented experiments involve the increase of flower production. Flower numbers in *Rhododendron indicum* ((L.) Sweet) cv Formosa increased

dramatically in response to increasing concentrations of foliar spray or soil drench PAC (Keever *et al.* 1990). In the same study, *Rhododendron* x *obtusum* (Az.) Clunt cv. Hino Crimson flower diameter was reduced with increasing concentrations of applied PAC. Alteration of floral morphology was also observed in kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson) where petal size and style length of flowers were reduced (Burge *et al.* 1990). In another study, the number of inflorescences per pot of *Hebe* x *francisciana* (Eastw.) Souster cv Variegata treated with a single foliar spray of PAC was doubled compared to control plants (Kristensen and Adriansen 1988). Finally, PAC also increased the numbers of inflorescences in separate male and female plants of buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.) (Yin and Quinn 1994).

Contradictory to such findings are an equal number of experiments in which PAC has had no significant influence on the numbers of flowers or inflorescences produced. Foliar spray and soil drench PA applied to zonal geranium (*Pelargonium hortorum* (L.H. Bailey)) had little effect on inflorescence numbers compared to control plants. Flowering was likewise unaffected by PAC applied to kiwifruit (*Actinidia deliciosa* (A. Chev) C.F. Liang et A.R. Ferguson) vines (Burge *et al.* 1990). In extreme situations PAC has been documented as actually depressing flower numbers in the case of Geraldton Wax (*Chamaelaucium uncinatum* Schaeur) treated with soil drench PAC (Dawson and King 1993).

4.5.2 Time to anthesis/precocity

The advance or delay of time to anthesis is another notable effect of PAC. Flowering was advanced by nearly three weeks in potted plants of *Hebe* x *francisciana* (Eastw.) Souster cv. Variegata treated with a single foliar spray of PAC (Kristensen and Adriansen 1988). Conversely, flowering precocity in *Eucalyptus nitens* (Dean et Maid.) ex Maid and *E. globulus* Labill. was unaffected by trunk injected PAC (Griffin *et al.* 1993). Time to anthesis in brown boronia (*Boronia megastigma* Nees) (Day *et al.* 1994) and zonal geranium (*Pelargonium hortorum* (L.H. Bailey)) (Tayama and Carver 1990) were unaffected by PAC while in staminate pistachio (*Pistachia vera* L. cv. B) anthesis was delayed by foliar application of PAC with the duration of delay proportional to the concentration of PAC used (Porlingis and Voyiatzis 1993).

4.5.3 Induction of flowering

PAC can also be substituted for physiological conditions required by some plants for flowering. PAC was found to be able to substitute for cool temperatures required for

flowering induction in white myrtle (*Hypocalymma angustifolium* Endl.) (Day *et al.* 1994) and, flowering was promoted in plants of *Gardenia jasminoides* Ellis cv. Veitchii, which is ordinarily a facultative short day plant (de Baerdemaeker *et al.* 1994).

At a more fundamental level the same study found that pollen production and pollen germination from pistachio was not affected by foliar sprays of PAC applied in the previous season of growth (Porlingis and Voyiatzis 1993).

4.5.4 Changes in sex expression

Perhaps the most curious influence of PAC on a plant has been the alteration of sex expression in grass flowers. Sex expression in inflorescences of monoecious buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm.) plants was found to be skewed towards femaleness when treated with foliar sprays of PAC (Yin and Quinn 1994). While GAs have been documented as promoting production of male flowers in cucumber (Cucumis sativus L.), PAC would suppress this expression (Roberts and Hooley 1988).

4.5.5 Summary

The promotion of floral induction by PAC in short day plants and those requiring cool temperatures is of significant interest. There is possbility for manipulation of growth without resorting to adjusting the physical environment of the plant being tested. This might be beneficial in the cut flower industry where large scale production of floral crops out of season could be advanced through judicious applications of PAC. The range of plants to be tested needs to be increased and encompass a range of floricultural crops. While PAC would never be a whole substitute for controlled environment growing conditions it might be used as a tool to promote simultaneous or asynchronous flowering for commercial markets. A similar prospect exists for manipulation of sex expression in flowers. While only tested on pistachio at this time, a range of monoecious nut or fruit crops might benefit from applications of PAC to synchronise flowering of staminate and pistillate trees, improving the chance of pollination.

4.6 Effects on seed / fruit

In some cases PAC has been shown to increase seed or fruit production while in others it has no effect or a negative effect. Examples of these experiments are briefly discussed here.

4.6.1 Changes in seed/fruit production

Mature avocado (*Persea americana* Mill.) trees were sprayed with a commercial formulation containing 25 % PAC. Increases in harvested fruits per tree resulted from spraying before or at the harvesting age compared to untreated plants (Adato 1990). The implication from this work is in enhancing the production of avocado trees, which are biennial bearers, during their 'off' season. However, in another study, Wolstenholme *et al.* (1990) found that yield and number of fruits in avocado cv. Hass and Fuerte were not significantly altered by treatment with foliar sprays of PAC.

In respect of the ineffectiveness of PAC in some species, Griffin *et al.* (1991) found that seed yield per capsule and seed germination were unaffected in two *Eucalyptus* species (*E. globulus, E. nitens*) which had been trunk injected with PAC.

In plants of *Ilex cornuta* cv. Burfordii Nana, fruit number actually decreased with increasing concentrations of soil applied PAC, yet increased when it was applied as a foliar spray (Keever *et al.* 1990). By contrast, foliar sprays of PAC on snapbean (*Phaseolus vulgaris* L.) resulted in plants producing a lower number of pods with a combined lower weight than control plants (El-Sayed 1991).

4.6.2 Seed germination

Finally, the effect of PAC on actual seed germination has only been poorly researched. In one documented experiment, PAC delayed the start, reduced the rate and decreased the percentage germination of 'Valencia' (*Citrus sinensis* (L.) Osbeck) orange seed during 4 months of observations (Bausher and Yelenosky 1987). Total germination was reduced by 50 % across the range of PAC concentrations used. Levels of ABA in wheat (*Triticum aestivum* L.) treated with soil drench PAC were found to decrease during a period 2 - 7 d after application, but resumed similar levels to that of controls after 14 d (Buta and Spaulding 1991). Levels of another natural plant hormone, GA_3, in apple seed from trees treated with PAC were initially half that of controls but after 3 y the level in treated and untreated seed was equivalent (Steffens *et al.* 1992).

4.6.3 Summary

The obvious benefit of PAC in some areas of fruit production is the possibility of enhancing fruit set and, ultimately, fruit size. However, there is little evidence to support a wide ranging research push into this area. It would appear that there are a myriad of factors to account for during fruit development and the majority of these

relate to environmental conditions (temperature, watering) and fertilising regimes. PAC has no influence, or very little influence on fruit development.

4.7 Conclusions

The prospect for further research into the effects of PAC on plants is generally promising. There is likely to be a move away from general experiments examining degrees of shoot growth inhibition into more specialised areas.

One of these is the area of plant tissue culture. PAC has had only a narrow exposure to this rapidly developing area of research and the possibilities for its use in promoting adventitious roots or inducing floral precocity are enormous. Production of plants from cuttings could also be enhanced with PAC for similar reasons although the flowering aspect might not be as critical.

The second promising area is in the synchronisation of flowering in monoecious crops. PAC could be used to synchronise flowering of staminate and pistillate plants enhancing the chances of pollination and possibly resulting in increased fruit set. Alternatively, PAC could be used to advance or delay floral induction to the requirements of the grower for the same purpose. Floral induction in commercial flower crops could produce plants ready for sale at most times of the year or to synchronise the flowering for sales during a planned marketing program.

Where PAC is likely to encounter problems as a researchable commodity is firstly, in its persistence and secondly, the possibility that a better growth inhibitor will be developed.

The persistence problem would be a headache for producers of floricultural crops desiring long flowering stems in a season following one in which other plants had been treated with PAC to reduce their size. In certain situations it may be beneficial (e.g. stunting or dwarfing conifers, creation of compact, 'bonsai' appearance plants for retail sale) but outside these specialised areas it would be a continuing problem. However, the problem of persistence can be reduced. All it requires is a careful application technique. Foliar sprays and soil drenching are the two most common methods of applying PAC to plants. The dispersal of the compound during these events could be minimised by shielding the spray, not spraying in the growing area and not allowing runoff from pots to contaminate benches or floors.

The issue of PAC being eventually superceded is unavoidable. However, PAC has the advantage of having a proven 'track' record, with experimentation extending back to the late 1970s. No other plant growth inhibitor has been as robust in surviving new developments and even the latest suite of synthetic growth inhibitors have been derived from triazole groups analogous to PAC (e.g. uniconazole). There is still plenty of scope for research with PAC, one could predict, for at least another decade.