

Modifying poppy growth and alkaloid yield with plant growth regulators

by

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ABSTRACT

Poppies (*Papaver somniferum* L.) are a major crop in Tasmanian broad-acre cropping rotations. Morphine has been the major alkaloid produced but now approximately half of the State's production is derived from thebaine-producing poppies. Yield potential in poppies is enhanced with earlier sowing, however this commonly results in excess vegetative growth and crop lodging. The effects of a range of plant growth regulators on plant growth and alkaloid yield of thebaine poppies were evaluated in three preliminary field trials conducted in northern Tasmania from 2002 - 04. Application of Slow Grow (maleic hydrazide) reduced seed weight in most trials and this result is potentially beneficial to industry as thebaine poppy seed is of limited commercial value due to thebaine residues. Application of Moddus (trinexapac) and Sunny (uniconazole) showed the greatest potential by altering alkaloid profile and reducing plant height respectively.

In a subsequent field trial with morphine poppies, single and split applications of Sunny across three growth stages were compared and while plant height was reduced, effects on plant lodging were inconsistent. Whereas lodging was decreased with the split application, the single application increased lodging, perhaps a result of the large height difference between main stem and lateral capsules. The split application of Sunny also resulted in a greater capsule yield compared with single and nil treatments through increased capsules/m². Despite a reduction in individual seed weight, seed yield from Sunny treatments was also greater due to a large increase in the number of seeds per capsule. It is proposed that the reduction in plant height and lodging with application of Sunny leads to reallocation of assimilates to yield components. Alkaloid content, in particular morphine, was also increased with application of Sunny and this is likely to be a result of extended alkaloid biosynthesis through delayed maturity.

The alkaloid, thebaine, is of higher value than oripavine and application of Moddus in three additional rate and timing trials consistently increased thebaine content. Associated with this, oripavine content decreased and this effect was rate responsive. In contrast with Sunny, Moddus had little effect on plant height or maturity. Instead, seed yield was reduced and straw yield tended to increase and combined with alkaloid effects, this consistently increased thebaine yield around 25%. Multiple and split

applications of Moddus tended to further increase thebaine content. As total alkaloid did not vary with single applications, it was postulated that Moddus inhibits the biosynthesis of oripavine from thebaine.

Results from this study and further trials conducted by industry have led to the registration of Moddus on poppy crops and close to 100% adoption rates by thebaine poppy growers. The potential of Sunny as a plant growth regulator (now marketed as Sumagic) has also been verified and in addition the benefit of split applications of both Sunny and Moddus demonstrated.

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CHAPTER 1. INTRODUCTION

Poppies (*Papaver somniferum* L.) are historically recognised for production of opioid drugs and were probably one of the earliest domesticated plants grown for medicinal value. The analgesic and narcotic properties of these plants have been known since the beginning of civilization (Duke, 1973; Hagel and Facchini, 2010). Poppy seed is also a valuable end product and is used for culinary purposes in bakery and confectionery industries (Duke, 1973; Bernath and Nemeth, 2009).

The history of poppy production in Australia and, in particular, Tasmania is relatively recent and has been discussed previously by Laughlin *et al.* (1998), Fist (2001) and more recently, Frappell (2010). Early production in Australia in the 19th century was restricted to medicinal use by doctors and recreational opium smoking by, in particular, Chinese immigrants (Laughlin *et al.*, 1998; Frappell, 2010). It was not until morphine shortages associated with World War II that more detailed investigations on morphine poppy production were conducted. However, plans for developing an industry were not seriously addressed until the early 1960s when a pharmaceutical subsidiary of the multinational Glaxo was keen to develop a more reliable source of opiate raw material than production from Western Asia and Eastern Europe (Laughlin *et al.*, 1998; Frappell, 2010).

Traditionally, alkaloids are extracted by manual lancing of the capsule wall and collecting the latex as raw opium. In 1928, the development of a break-through method to extract alkaloid from mechanically harvested dry poppy straw significantly reduced labour costs enabling broad-acre morphine poppy production (Hagel *et al.*, 2007). Out of season production and a relatively dry harvest period over summer compared with England were key criteria in selecting Australia as a potential supplier of poppy opiates (Laughlin *et al.*, 1998). With cooperation from the local Department of Agriculture, field trials in Tasmania were commenced in 1960 with Glaxo (now GlaxoSmithKline) beginning commercial production in the late 1960s (Frappell, 2010). Poppy production in the State further expanded in 1975 with the establishment of Tasmanian Alkaloids, this company being subsequently purchased by Johnson and Johnson (Fist, 2001;

Frappell, 2010). A third local company, TPI Enterprises, commenced operations in 2004 (TPI, 2010).

Poppies are now an important crop in Tasmanian broad-acre cropping rotations and one of the State's major agricultural commodities with a farm gate value of between \$60 and \$80 million (Frappell, 2010). Through focused research and development (as well as effective extension practices), Tasmania is now considered the world's most efficient international producer of poppies with the highest yield per hectare of opiate-producing countries (PACB, 2010). Tasmania has a number of cultural advantages in maintaining this leading edge: the temperate climate is generally not conducive to serious poppy diseases or pests; long daylight hours increase floral initiation and crop yield (Frappell, 2010); the harvest period over summer is relatively dry ensuring high alkaloid content; and the industry has assembled considerable skills and intellectual capacity. In addition, one strategic advantage with poppy production in Tasmania is the island isolation and capacity to control illegal activities. Licit poppy production is monitored and controlled by the International Narcotic Control Board (INCB) with powers to reduce and expand supply depending on existing global production (Hagel *et al.*, 2007; Laughlin *et al.*, 1998). Australia, as a signatory to the 1961 Single Convention on Narcotic Drugs, abides by these supply requirements and hence production is tightly regulated. Licences to grow poppies in Tasmania require security clearances from the State controlled Poppy Advisory and Control Board (PACB). Poppy crops are regularly monitored during the growing season and post-harvest operations are required to ensure suitable treatment of harvest residue and poppy re-growth (Laughlin *et al.*, 1998; PACB, 2010).

The estimated area of poppy production in 2010 in Tasmania is over 24 000 ha (INCB, 2009) but increased demand for land has resulted in production moving to less well drained soils that are more prone to restricted root growth from water-logging (Chilvers, 1996). Farming systems using raised beds were introduced into Tasmania in the late 1990's to help alleviate water-logging. While this has enabled establishment of poppies in winter and early spring (Fist, 2001), earlier sowing commonly results in excess vegetative growth and subsequent lodging towards the end of the season. Furthermore, refinements in general poppy crop nutrition, disease control and irrigation have led to increased yield potential but also additional crop growth and greater potential for crop lodging.

Lodging in poppies results in losses in capsule and seed yield (Nagy *et al.*, 1989; Kumar *et al.*, 2010) and annual losses in Tasmania have been estimated at 10% of the total crop (A. Fist pers. comm.). Impacts on alkaloid yield and content are particularly large where poppy crops are mechanically harvested. As alkaloid in poppies is concentrated in the capsule (Chung, 1987; Srivastava and Sharma, 1990) and processing costs are higher for low alkaloid material (Fist, 2001; Larkin *et al.*, 2007), the harvest operation aims to remove only the capsules, with minimal stem. The height of capsules from lodged plants is lower than the harvester front and these capsules remain unharvested (Laughlin, 1987; Chung, 1990). Lodged poppy crops have on occasions been harvested using conventional grain harvesters fitted with pick-up fronts but the operation is considerably slower resulting in increased production costs (A. Fist pers. comm.). Lodging also increases the risk of fungal attack and this can reduce alkaloid content (Nagy *et al.*, 1989).

Crop lodging occurs in a number of agricultural and horticultural crops and the literature review in Chapter 2 provides an overview of causes, impacts and strategies to reduce lodging. In particular, application of plant growth regulators (PGRs) has decreased the incidence of lodging in other crops and the following literature review focuses on currently used plant growth regulators, their effects on plant hormones, growth and development and variability of responses. As there is considerable previous literature on lodging in cereal crops and little information on poppies, the emphasis is on cereals with reference to poppies where applicable. To set the scene, there is an initial general discussion of poppy cultural requirements and, as trial work encompassed different poppy alkaloids, a review of alkaloid biosynthesis.

CHAPTER 2. LITERATURE REVIEW

2.1 Poppy production and alkaloid biosynthesis

2.1.1 Introduction

Poppies are dicotyledonous annual herbs belonging to the family *Papaveraceae* and generally grow to a height of 1.4 m. Poppies have four generally pale pink petals with a dark basal blotch. The capsules are spherical with a flat cap, 20-40 mm in diameter and contain a large number of very small seeds (Mahdavi-Damghani *et al.*, 2010). Poppies differ from most crops as the capsule, being the major site of alkaloid deposition, is the most economically important component of the plant at maturity (Chung, 1987; Srivastava and Sharma, 1990). Poppy crop development has been broadly divided by Mahdavi-Damghani *et al.* (2010) into 6 stage s: (1) seedling; (2) rosette leaves; (3) budding (hook stage); (4) anthesis; (5) technical maturity (ready for lancing); and (6) biological maturity (ripening of dry seed). However this index provides insufficient detail of the growth stages at the time that crop chemicals are applied and poppy development is better represented in Figure 2.1 below.

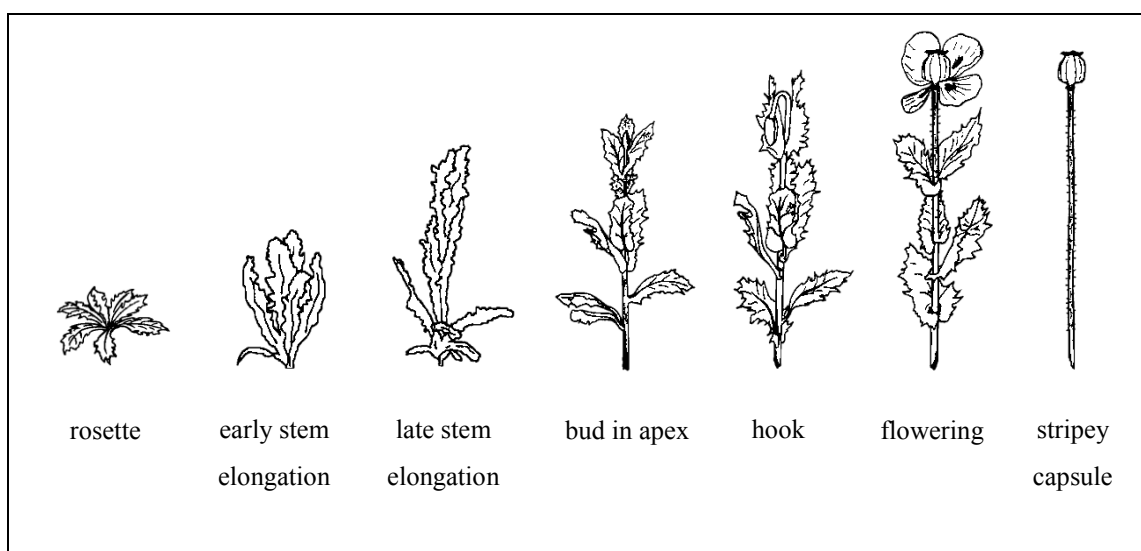


Figure 2.1. Poppy growth stages. Diagrams provided courtesy of Tasmanian Alkaloids Pty Ltd.

2.1.2 Cultural requirements

Poppies require a free draining soil and initial production in Tasmania was centered on well structured ferrosol soils of the north-west coast (Laughlin *et al.*, 1998; Frappell, 2010). Poppies also require a soil preferably above pH (H₂O) 5.7 (Temple-Smith *et al.*, 1983) and planting generally occurs in more intensive rotations where liming of the soil is common place. To enable high crop yields, adequate basal soil nutrition is required, particularly nitrogen (N) and phosphorous. Later in the season, alkaloid content is generally very responsive to top-dressed N (Laughlin, 1983; Fist, 2001). However without good soil moisture, responses to N may be limited (Laughlin and Chung, 1992) and consequently irrigation is generally applied to commercial crops.

Poppy crop yields and thus grower adoption were initially slowed by narrow herbicide options for control of broad leaf weeds (Fist, 2001; Frappell, 2010). However, continuous and extensive evaluation of a range of herbicide and adjuvant products has subsequently resulted in effective control of most weeds. Herbicides currently registered for use in poppies include diquat (Reglone), clomazone (Command), metosulam (Eclipse) and a range of grass selective herbicides. Diquat is classified as a non-selective herbicide but due to the surface characteristics of poppy leaves, it causes little damage (Fist, 2001). Disease and pests have caused concern but are generally restricted with an adequate control program. The most common disease is downy mildew (*Peronospora cristata*) (Dennis, 1998; Laughlin *et al.*, 1998) and two to three fungicides are generally applied from stem elongation to flowering. Problems with pests generally relate to early seedling growth stages, in particular slugs (generally *Deroceras spp.*) and springtails (*Collembola spp.*) (Dennis, 1998) that have increased in prominence with trends toward minimum cultivation and stubble retention. While baiting with a range of molluscicides is generally effective against slugs, pre-sowing incorporation of insecticides to control springtails is considerably more expensive (Laughlin *et al.*, 1998). European skylarks (*Alauda arvensis*) can also be problematic at establishment by destroying emerging seedlings (Dennis, 1998) but the sporadic nature of damage makes control difficult.

2.1.3 Poppy alkaloids and biosynthesis

Alkaloids are secondary plant metabolites that evolved as defense against predation by animals, insects and pathogens (Zenk and Juenger, 2007). Different plant families have developed various secondary metabolites and it has been estimated there are 12 000 known alkaloids with a number of these used as medicines, pharmaceuticals and poisons (Ziegler and Facchini, 2008). Alkaloids contain a nitrogenous base and are often classified on their amino acid precursor (Dewick, 2002). Tyrosine is the base of a number of alkaloids including the benzyloisoquinoline (BIA) group formed in poppies. There are approximately 2500 BIA alkaloids across several plant families but morphine and codeine are only produced in opium poppies (Hagel *et al.*, 2007; Ziegler and Facchini, 2008).

The biosynthesis of morphine from tyrosine has been extensively studied beginning with determination of pathway precursors, characterization of individual enzymes and, more recently, isolation of genes encoding many of the enzymes (Zenk and Jeunger, 2007; Hagel and Facchini, 2010). The key reactions will be discussed briefly with emphasis on the morphinan pathway that leads directly to production of morphine. A simplified scheme of alkaloid pathways in opium poppies is shown in Figure 2.2, adapted from Frick *et al.* (2005) and Hagel *et al.* (2007). For further detail, see papers by these authors and also Dewick (2002).

From condensation of two tyrosine molecules, there are 19 chemical steps to the formation of morphine as well as two major branch pathways diverging from the key intermediate (S)-reticuline (Frick *et al.*, 2005; Hagel *et al.*, 2007). Conversion to (S)-scoulerine leads to biosynthesis of two alkaloids, sanguinarine and noscapine. Sanguinarine which is generally found in higher levels in the roots possesses anti-microbial activity (Hagel *et al.*, 2007) and is sometimes used in oral hygiene products (Ziegler and Facchini, 2008). Noscapine is an effective cough suppressant (Dewick 2002; Ziegler and Facchini, 2008).

The first committed steps in the morphinan pathway are via (R)-reticuline leading to biosynthesis of thebaine. Derivation of morphine from thebaine occurs through demethylation to form neopinone and then codeine (Zenk and Jeunger, 2007; Hagel *et*

Addiction to morphine produces a dependency that is difficult to overcome. Consequently other opioids that are less addictive such as codeine are commonly synthesized from morphine. A number of fully synthetic opioid drugs such as pethidine and methadone are structurally similar to morphine but generally with fewer side effects (Dewick, 2002). Thebaine can be synthesized into codeine and opioids such as oxycodone, buprenorphine and naltrexone (Fist, 2001). Some of these drugs can be up to 10 000 times more potent than morphine and are used to tranquilise large animals while others are analgesics without the narcotic and other side effects of morphine (Dewick 2002; Millgate *et al.*, 2004).

As heroin is more efficiently derived from morphine, replacing morphine producing crops with those producing thebaine as the raw material is seen as an effective strategy to combat illicit opium trade (Fist, 2001; Dewick 2002). However, as thebaine is present at low levels in morphine crops, it has been uneconomic to extract and has been regarded until recently as being of limited value. International interest in developing thebaine producing crops initially focused on an alternative species, *P. bracteatum* that only produces thebaine. However, after screening of mutagen-treated seedlings a *P. somniferum* plant was discovered that accumulates thebaine and oripavine instead of morphine and codeine (Fist, 2001; Millgate *et al.*, 2004). This mutant known as “*top1*” (thebaine oripavine poppy 1) carries a single recessive gene and has now been used extensively in breeding material (Fist, 2001; Hagel *et al.*, 2007). In the *top1* mutant, demethylation of thebaine to neopinone and in the alternative pathway, oripavine to morphinone is inhibited (Millgate *et al.*, 2004) and recently this has been attributed to lack of a 2-oxoglutarate-dependent dioxygenase, codeine O-demethylase, (Hagel and Facchini, 2010). Using similar techniques, Tasmanian Alkaloids have further developed the “Ted” mutant in which it is proposed both codeine O-demethylase and another dioxygenase, thebaine 6-O-demethylase are inhibited resulting in almost exclusive thebaine biosynthesis (A. Fist pers. comm.).

In conclusion the poppy industry in Tasmania, in particular the private poppy sector, has been very successful in breeding for higher crop yields and alkaloid content, generally through conventional crossing and selection but also through alternative strategies such as mutagenesis. The average crop yield in Tasmania is now around 2.5 t/ha (PACB, 2010) and average alkaloid content ranges from 1.5 to 2.7 % dry weight (Allen *et al.*,

2008), a two to four-fold increase over the last 20 years (A. Fist pers. comm.). In particular, the development of the *top1* mutant has revolutionized the poppy industry in Tasmania and the thebaine crop now contributes 50 % of the State's total poppy alkaloid production. Advances in biotechnology are also being considered for altering other steps in alkaloid pathway biosynthesis. Developing a codeine-producing poppy variety by inhibiting the conversion of codeine to morphine was suggested as a strategy to remove the need for morphine-producing crops (Hagel and Facchini, 2010) and the world's first codeine chemotype "Tasman" has been recently released in Tasmania (Williams, 2009). Other researchers are also examining metabolic engineering as a means of changing enzyme activity or creating over expression of enzymes involved in rate-limiting steps (Frick *et al.*, 2007; Larkin *et al.*, 2007).

2.2 Crop lodging

2.2.1 Introduction

Lodging is defined as the state of permanent displacement of plant stems from the upright position (Pinthus, 1973). There are different forms of lodging and Pinthus (1973) has categorised these as either stem lodging where the lower internodes buckle or root lodging in which the anchorage of the plant fails. There has been some conjecture as to the predominant form of lodging in cereals and this may partially relate to plant stature with modern semi-dwarf germplasm and a smaller root:shoot ratio being more prone to root lodging than older tall varieties (Zuber *et al.*, 1999). Berry *et al.* (2004), in a review of lodging in cereals suggest that both forms occur in the paddock and that the confusion is probably due to the difficulty in observing the lower stems of lodged crops, being covered with other fallen plants. Stem lodging mostly occurs through storm damage but can increase in prevalence in cereals with stem diseases (Berry *et al.*, 2000), in particular eyespot, *Pseudocercospora herpotrichoides* (Pinthus, 1973) or at early growth stages in highly-tillered crops (Fischer and Stapper, 1987). Unless caused by disease, stem lodging generally only occurs when the soil is dry and unyielding (Pinthus, 1973). In contrast, root lodging is common after rain when the torque created by the wind weakens plant anchorage (Baker *et al.*, 1998) and causes the stem to overturn or rotate about its base (Pinthus, 1973).

There are no published reports citing the type of crop lodging that occurs in poppies; however, Nagy *et al.* (1989) refer to damage from strong winds causing plants to lodge and break, suggesting stem lodging. It is likely that stem lodging is also the predominant form in Tasmania with stems commonly breaking at around 15 cm from the base or lateral branches bending over and often detaching from the main stem (A. Fist pers. comm.). In previous studies evaluating mechanical lodging of poppy plants at maturity, there was no root lodging and stems tended to bend or snap (Dean, unpublished). Some root lodging has however been observed and is probably more common after high rainfall events (particularly after irrigation) followed by strong winds.

2.2.2 Crop losses from lodging

Lodging impacts on crop growth and development through reduced water and nutrient translocation, particularly if due to stem buckling and increased disease levels arising from greater humidity (Tripathi *et al.*, 2003). Greater shading also affects light interception with reduced carbohydrate assimilation (Pinthus, 1973). Grain yield losses of 50% have been reported (Syme, 1968; Lowe and Carter, 1970; Batch, 1981) and up to 80% (Easson *et al.*, 1993). The timing of lodging greatly influences the extent of losses (Tripathi *et al.*, 2004). Fischer and Stapper (1987) induced lodging in wheat at different growth stages and greatest yield losses occurred in the period from anthesis to 20 days post-anthesis, largely due to reduced grains/ear. Yield losses were less with pre-anthesis lodging due to the crop being able to stand back up as later elongating internodes grew upright. Lodging during late grain filling was also less damaging but resulted in a 7% reduction in 1000 grain weight. Batch (1981) similarly recorded a yield loss of 50% in wheat with early lodging compared with 11% where lodging occurred towards maturity. The severity of lodging also influences yield loss. In the study by Fischer and Stapper (1987), induced lodging at 80° from vertical decreased yield 17% compared with a 9% loss at a 45° angle. In cereals, the upper leaves are most critical for photosynthesis and, if unaffected, there will be reduced yield loss (Fischer and Stapper, 1987). Conversely, lodging at maturity may increase yields through less movement in the wind, resulting in decreased grain shedding and shattering losses as reported in canola (Baylis and Hutley-Bull, 1991; Kightley, 2001) and ryegrass (Borm and van den Berg, 2008).

Lodging also affects the harvest operation through reduced ease of harvest, yield losses and potential drying expenses (Pinthus, 1973; Foster and Taylor, 1993, Berry *et al.*, 2004) and can additionally impact on crop quality parameters. Small grain size is undesirable for malting barley in particular, and in the study of Fischer and Stapper (1987), the more humid environment under the lodged crop led to grain sprouting. As discussed in Chapter 1, impacts of lodging on alkaloid content in poppies can be particularly problematic when harvested mechanically.

2.2.3 Plant characteristics associated with reduced lodging

2.2.3.1 Stem characteristics

A number of stem characteristics influence lodging in cereals, the most obvious being stem height. The centre of gravity is higher in taller varieties and increases with stem elongation after anthesis through to grain-fill until the crop begins to senesce (Crook and Ennos, 1995; Berry *et al.*, 2000). Studies comparing varieties and agronomic treatments among cereals have demonstrated positive correlations between plant height and lodging in wheat (Wiersma *et al.*, 1986; Crook and Ennos, 1995; Zuber *et al.*, 1999; Tripathi, *et al.*, 2003) and barley (Foster and Taylor, 1993). The association is however not clear cut and other studies in wheat have found a less strong correlation (Batch, 1981; Easson *et al.*, 1993).

In many studies, stem characteristics other than height have proven more useful in predicting susceptibility to lodging, in particular stem diameter in wheat (Easson *et al.*, 1993; Zuber *et al.*, 1999; Tripathi *et al.*, 2003) and sunflowers (Hall *et al.*, 2010). More detailed studies have also reported strong correlations between lodging and stem weight per unit length (Zuber *et al.*, 1999), stem wall thickness (Tripathi *et al.*, 2003; Hall *et al.*, 2010) and ratio of stem wall thickness to stem radius (Wang *et al.*, 2006). Differences in plant anatomical and chemical structure and their effect on lodging have also been investigated although results have generally been inconsistent. Lodging resistance has been related to a higher number of vascular bundles in the stems of cereals (Pinthus, 1973). However, Zuber *et al.* (1999) in a study of the association of lodging with 29 traits in wheat reported a poor correlation with number of vascular bundles. More recently Wang *et al.* (2006) determined that the location and size of vascular bundles was important with a high number of large vascular bundles in parenchyma tissue leading to increased resistance to bending. Higher lignin content has also been associated with reduced lodging (Pinthus, 1973). However, Knapp *et al.* (1987) concluded that lodging was not associated with level of lignin and structural carbohydrates content in the stem wall or water soluble carbohydrates in the stem. Wang *et al.* (2006) also found no correlation with lignin but reported lower cellulose content in wheat varieties with low bending strength. It is possible some of this variability may be due to the effect of different growing conditions on plant growth. For example, under drought conditions, stem water soluble carbohydrate level increases in

response to the environment rather than being constitutive (Pierre *et al.*, 2010). Likewise, as stem strength of plants is greater in exposed conditions compared with sheltered (Cleugh *et al.*, 2008), this characteristic is also largely responsive to the environment and may only be partially under genetic control.

2.2.3.2 Root characteristics

Root characteristics associated with lodging resistance generally relate to greater root vigour (Pinthus, 1973) and in particular the number, length and spread of coronal roots in wheat (Pinthus, 1973; Crook and Ennos, 1995). Total root dry weight does not accurately reflect the extent and spread of root development (Pinthus, 1973) although dry matter (DM) measurements partitioned by depth are more accurate (Emam and Shekoofa, 2009). Berry *et al.* (2000) concluded that greater root spread is also associated with high tiller numbers and this has the effect of greater plant leverage forces. Tripathi *et al.* (2003) also reported a strong positive correlation between the total number of tillers and lodging.

To overcome difficulties and time constraints in measuring root and stem characteristics, mechanical tests for stem strength or bending resistance that impose force on a defined length of stem material have been developed (Pinthus, 1973; Crook and Ennos, 1995; Baker *et al.*, 1998; Berry *et al.*, 2000). Artificial methodology has also been developed to simulate lodging and these deliver a pushing rotational force to plant stems (Crook and Ennos, 1995; Berry *et al.*, 2003). The latter test is field based and measures the required force to push a row of plants beyond the vertical axis. Results have been comparable with lodging scores and variety rankings from field trials. Similar artificial lodging tests have also been employed more recently to show variation in sunflower germplasm (Sposaro *et al.*, 2008).

2.2.4 Lodging and crop management

As outlined in previous studies (Pinthus, 1973; Crook and Ennos, 1995; Berry *et al.*, 2000; 2004), crop management practices can reduce the severity of lodging through lower stem height (reduced nitrogen, delayed sowing, PGRs) and increased stem strength (delayed sowing, reduced nitrogen). Crook and Ennos (1995) recorded large reductions in stem and anchorage strength with additional nitrogen, the latter due to

fewer and less rigid coronal roots. In other studies, rate of nitrogen has not changed root DM but with a greater response in shoots, the root: shoot ratio is reduced leading to increased lodging in wheat (Pinthus, 1973). As discussed by Berry *et al.* (2000), reduced sowing rate in cereals will increase root lateral spread and anchorage but this is partially offset by a greater number of tillers/plant and thus increased plant leverage.

Wet upper leaf surfaces will increase the centre of gravity and overturning forces (Berry *et al.*, 2004) and thus irrigation can be expected to increase lodging (Pinthus, 1973). Irrigation, in particular over-watering, will also increase the incidence of root lodging through wet soil (Pinthus, 1973). In contrast, increased soil strength and bulk density associated with minimum tillage has reduced the incidence of root lodging (Berry *et al.*, 2004). In a number of studies application of PGRs, through effects on plant growth and development, has proven an effective management tool in significantly reducing crop lodging and the remainder of this review examines the use and effects of PGRs.

2.3 Use and effects of plant growth regulators

2.3.1 Introduction

Plant growth regulators (PGRs) mimic or affect production of plant hormones and are thus able to modify plant growth and development, this being a regulatory rather than a nutritional role (Machakova *et al.*, 2008). They are generally only applied at low rates without showing phytotoxic effects (Rademacher and Brahm, 2010). Although there are a number of natural PGRs (see review by Gross and Parthier, 1994), effects have generally been small and inconsistent and this review focuses on synthetic PGRs.

The first synthetically derived PGR applied to wheat, chlormequat (CCC), was found to decrease height and increase stem diameter (Tolbert, 1960). With cereal crop varieties in the 1960s being predominantly tall in stature, subsequent studies focused on the ability of CCC to reduce lodging (Humphries *et al.*, 1967; Syme, 1968; Lowe and Carter, 1970). Another PGR, ethephon (ETH) achieved similar results and was widely tested in cereals in the 1980s (Batch, 1981; Nafziger *et al.*, 1986; Waddington and Cartwright, 1986; Wiersma *et al.*, 1986). Early work with PGRs was also able to demonstrate yield increases in the absence of lodging and, in particular, several studies highlighted that early application of CCC during the tillering phase of cereal development resulted in greater tiller production and subsequently higher yields (Tolbert, 1960; Humphries *et al.*, 1967).

Concurrent with research into PGRs, the international wheat breeding focus in the 1960s was largely directed to introducing dwarfing genes into local varieties to produce shorter stature, high yielding varieties with superior standing ability and Syme (1968) suggested that more attention should focus on this approach to control lodging. With reduced tendency to lodge, subsequent studies largely focused on earlier application of PGRs to influence yield components in wheat and barley. More recently with a trend towards earlier sowing dates and the general adoption of complete crop management packages, in particular higher nitrogen and fungicide inputs, crop lodging has again become more problematic and the use of PGRs commonly beneficial. Although there may not always be a yield advantage, this strategy is very effective insurance against losses if lodging is severe (Batch, 1981; Rademacher, 2000).

New PGRs, termed by Grossman (1992) as second generation (in particular the triazoles) and third generation (acylcyclohexanedione) PGRs that display increased specificity, have also been developed over the last two decades. Plant growth regulators and suppressants are now applied in a number of agricultural and horticultural crops to reduce vegetative growth and potentially modify yield, its components and quality. Table 2.1 lists some of the current PGRs, major crop usage and abbreviations used in this thesis.

Table 2.1. Common names, abbreviations and major crop usage of PGRs and suppressants applied in field trials.

Trade name	Common name	Abbrevn used	Active ingred.	Type of chemical (and major crop use)
Apogee ⁺	prohexadione	Pro-Ca	275 g/kg	PGR (apples)
Caramba	metconazole	MET	90 g/L	PGR (canola*)
Cultar	paclobutrazol	PBZ	250 g/L	PGR (apples, mango, stone fruit)
Cycocel	chlormequat	CCC	770 g/L	PGR (cereals)
Eclipse	metosulam		714 g/L	Herbicide (cereals, lupins, poppies)
Ethrel	ethephon	ETH	480 g/L	PGR (barley, apples, sugar cane)
Folicur	tebuconazole	TEB	430 g/L	Fungicide (cereals), PGR (canola*)
Moddus ⁺⁺	trinexapac	TE	250 g/L	PGR (wheat*, ryegrass, sugar cane)
Opus	epoxiconazole		250 g/L	Fungicide (cereals)
Pix	mepiquat	MEQ	38 g/L	PGR (cotton, barley*)
Slow Grow	maleic hydrazide		270 g/L	Growth suppressant (potatoes, onions)
Sunny**	uniconazole	UNI	50 g/L	PGR (avocadoes**)

⁺ new formulation is Regalis; * not registered in Australia;

⁺⁺ also sold as Primo in turf grass, now also a permit for use in poppies;

** now registered with same formulation as Sumagic for use in poppies.

2.3.2 Effects of plant growth regulators on plant hormones and secondary metabolites

There are two main groups of PGRs, inhibitors of gibberellic acid (GA) biosynthesis and ethylene producing compounds (Grossman, 1992; Rademacher, 2000). Most commercial PGRs act by inhibiting GA formation with different classes of anti-GA

PGRs blocking activity of specific enzymes. While the main effect of these PGRs is on GA metabolism, the isoprenoid pathway in plants also generates common precursors for biosynthesis of ABA, cytokinins, carotenoids and terpenoids and thus impacts of PGR application on plant growth and development are many and varied.

2.3.2.1 Gibberellic acid metabolism

GA controls many developmental aspects of plant growth with major roles in promoting stem extension, inducing germination in the seed, bolting of rosette plants and enabling fruit set and growth (Rademacher, 2000; Kim *et al.*, 2007; Bhattacharya *et al.*, 2010). To date, 136 GAs have been isolated (Bhattacharya *et al.*, 2010) but it appears no plant has all GAs (Moshkov *et al.*, 2008). GAs are classified by a gibberellin number (GA_x) rather than by chemical structure. The majority are precursors and biologically inactive with the most bioactive form in most plants being GA₁ (Moshkov *et al.*, 2008; Bhattacharya *et al.*, 2010). The largest effects of anti-GA PGRs are associated with changes in endogenous GA levels. Evidence of PGR effects on GA metabolism is widespread and application of anti-GA PGRs generally results in reduced shoot growth across a range of crop plants and, where measured, a decrease in the level of GA₁ (Rademacher, 2000; Kim *et al.*, 2007). Associated with this is a parallel increase in GA₂₀, commonly the penultimate inactive precursor (Adams *et al.*, 1992; Rademacher *et al.*, 1992). This effect has also been shown to be rate responsive (Kang *et al.*, 2010).

The biosynthesis of GA has been reviewed by Rademacher (2000), Fletcher *et al.*, (2000) and more recently Yamaguchi (2008). A simplified scheme of the isoprenoid pathway leading to GA biosynthesis adapted from Fletcher *et al.*, (2000) and Rademacher (2000) is shown in Figure 2.3. There are three main stages. The first commences with mevalonic acid and other metabolites (Rademacher, 2000; Bhattacharya *et al.*, 2010). Key intermediates in this phase are isopentenyl diphosphate (IPP), farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) that are also precursors for cytokinin, abscisic acid (ABA), sterol, terpenoid and carotenoid biosynthesis (Grossman, 1992; Fletcher *et al.*, 2000). The onium-type PGRs such as CCC and MEQ inhibit two cyclisation steps in the transformation of GGPP to *ent*-kaurene (Grossman, 1992; Rademacher, 2000).

The second stage of GA biosynthesis is characterized by a series of oxidation reactions resulting in GA₁₂-aldehyde, the first intermediate specific for GA production (Fletcher *et al.*, 2000; Rademacher, 2000). The conversion of *ent*-kaurene to *ent*-kaurenoic acid is catalysed by cytochrome P₄₅₀-dependent monooxygenases (Grossman, 1992). Triazole compounds such as PBZ and UNI have a similar structure to *ent*-kaurene and *ent*-kaurenoic acid and are highly efficient in binding and inactivating these enzymes (Rademacher, 2000). Many of the triazoles occur as different enantiomers with the (S) form being more inhibitory of GA biosynthesis and (R)-enantiomers possessing greater effect on sterol biosynthesis and consequently stronger anti-fungal properties (Rademacher, 2000).

In the final stage, bioactive gibberellins, in particular GA₁, are formed after oxidation of a number of biologically inactive forms of GA. Several of these reactions are catalysed by dioxygenases (Rademacher, 2000; Rademacher *et al.*, 2006; Mutasa-Gottgens and Hedden, 2009) and to maintain GA homeostasis, another variation of this enzyme deactivates GA₁ to the inactive GA₈ form (Rademacher, 2000; Achard and Genschik, 2009; Bhattacharya *et al.*, 2010). Dioxygenases require 2-oxoglutaric acid as a co-substrate and acylcyclohexanedione PGRs such as Pro-Ca and TE being structurally similar to this compound, inhibit GA biosynthesis (Adams *et al.*, 1992; Rademacher, 2000; Kang *et al.*, 2010). Catabolism of active GA is also blocked by the same complex and application of acylcyclohexanedione PGRs have thus resulted in increased stem elongation in *Matthiola incana* L. (Hisamatsu *et al.*, 1998) and pod length in field peas (Sponsel and Reid, 1992). Rademacher *et al.* (2006) consider this effect is dependent on shoots being actively growing at the time of application, presumably during a period of high rate of active GA biosynthesis with less effect from inhibition.

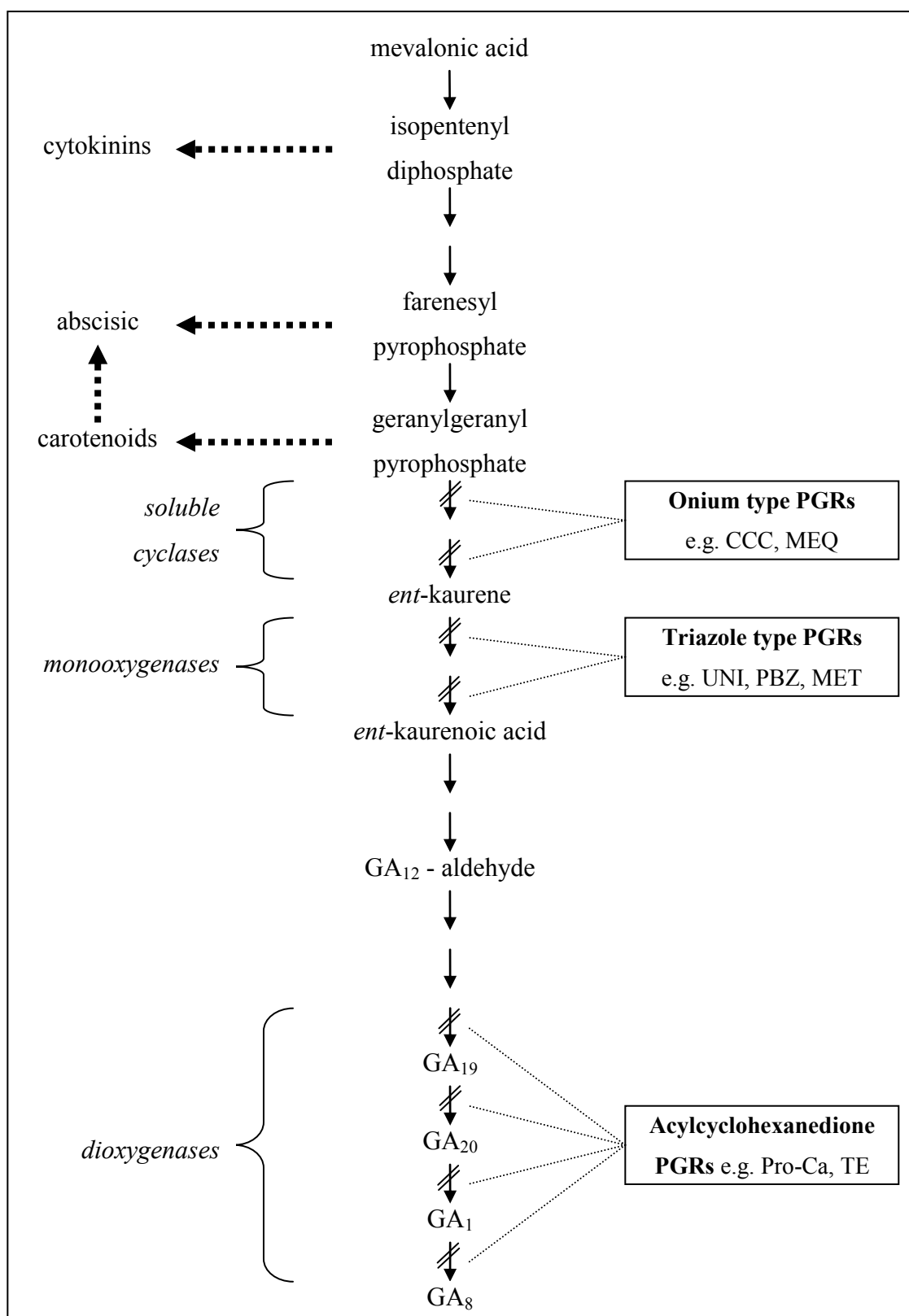


Figure 2.3 Schematic representation of the isoprenoid GA biosynthesis pathway and side pathways. Anti-GA PGRs that inhibit steps are shown in bold and the respective enzymes in italics. Adapted from Fletcher *et al.* (2000) and Rademacher (2000).

2.3.2.2 Absciscic acid metabolism

ABA is commonly known as a stress hormone and is recognized for increasing plant adaptability and tolerance to cold, drought and other environmental stresses. ABA is also implicated in inhibition of seed germination, delay of flowering in long day plants and inducing dormancy in the shoots of deciduous trees (Fletcher *et al.*, 2000; Moshkov *et al.*, 2008). A number of studies across a range of plants have shown increased levels of endogenous ABA after application of triazole (Hauser *et al.*, 1990; Grossman, 1992; Chizhova *et al.*, 2005) and acylcyclohexanedione PGRs (Grossman *et al.*, 1994; Bingham and McCabe, 2006).

As shown in Figure 2.3, ABA can be formed directly from FPP or indirectly from the conversion of GGPP to carotenoids (Fletcher *et al.*, 2000). In addition to GA biosynthesis, P₄₅₀-dependent monooxygenases are also responsible for catabolism of ABA to the biologically inactive phaseic acid (Hauser *et al.*, 1990; Moshkov *et al.*, 2008) and triazole PGRs are thus effective in inhibiting this reaction (Grossman, 1992). The response in ABA level after application of triazole PGRs is rapid and precedes morphological changes (Chizhova *et al.*, 2005) but also transitory with a subsequent sharp decline (Hauser *et al.*, 1990). High triazole application rates have also decreased ABA levels (Hauser *et al.*, 1990; Grossman *et al.*, 1991) and a biphasic response has been proposed with lower ABA levels caused by either newly stimulated catabolism or inhibition of biosynthesis (Hauser *et al.*, 1990). Response to acylcyclohexanedione PGRs is through a different mechanism as elevated ABA levels were maintained with high concentrations of Pro-Ca and, according to Rademacher (2000), effects on ABA are likely to be indirect. It is possible inhibition of GA biosynthesis increases flux of GA precursors leading to enhanced ABA biosynthesis (Figure 2.3).

Higher levels of endogenous ABA after triazole PGR application have been accompanied by a proportional decrease in stomatal opening, water use and transpiration rate (Hauser *et al.*, 1990; Grossman, 1992; Fletcher *et al.*, 2000). In addition to reduced dry matter accumulation, these morphological effects are characteristic of drought stress (Farooq *et al.*, 2009) and it is also well documented that increases in ABA are triggered by dehydration of plant tissue (Moshkov *et al.*, 2008) and low soil moisture (Outlaw *et al.*, 1992; Gianfagna *et al.*, 1992). Thus PGRs, through

mediating affects on ABA level, can influence water balance within the plant (Hauser *et al.*, 1990; Fletcher *et al.*, 2000; Bingham and McCabe, 2006).

Through increased levels of endogenous ABA and similar effects on water use, triazole PGRs have also been implicated in improved cold hardiness (Gianfagna *et al.*, 1992; Grossman, 1992). It has been suggested that low temperature accelerates degradation of membrane lipids through an increase in oxygen free radicals (Fletcher *et al.*, 2000). As triazoles enhance plant oxidant potential (Fletcher *et al.*, 2000; Jaleel *et al.*, 2006), increased tolerance to cold may be a function of reduced oxidative injury and improved membrane integrity (Fletcher *et al.*, 2000; Baninasab, 2009). Triazole application has also resulted in increased tolerance to heat (Gilley and Fletcher, 1997; Fletcher *et al.*, 2000) and herbicide damage (Gilley and Fletcher, 1997) and it is likely this is similarly through improved anti-oxidant activity. Acylcyclohexanedione PGRs have also shown similar stress protection benefits with reduced flower damage in apples from frost (Albrecht *et al.*, 2004). An increase in anti-oxidant properties is again implicated with generation of a higher level of flavonoid compounds suggested to enhance frost tolerance (Rademacher *et al.*, 2006).

2.3.2.3 Ethylene metabolism

Ethylene is involved with regulation of developmental processes including fruit ripening, breaking of dormancy in seeds and buds and defence-related proteins in plants (White *et al.*, 1992; Morgan and Drew, 1997; Moshkov *et al.*, 2008). The effects of ethephon in retarding plant growth have been recognised since the 1960s (Yang, 1969). Ethephon degrades in plant tissue to form ethylene (Yang, 1969; Moshkov *et al.*, 2008) that inhibits cell elongation (Rajala *et al.*, 2002). However there is conjecture as to whether ethylene acts directly through influence on other plant hormones such as auxin (Woodward and Marshall, 1988) or ethephon is transported within the plant with subsequent release of ethylene at the target organ (Foster and Reid, 1992).

Ethylene is formed from methionine with intermediates of S-adenosyl methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Foster and Reid, 1992; Moshkov *et al.*, 2008). Plant ethylene concentration is also influenced by anti-GA PGRs with biosynthesis inhibited by both triazole (Grossman, 1992; Fletcher *et al.*, 2000; Rademacher, 2000) and acylcyclohexanedione PGRs (Rademacher, 2000; Kang *et al.*,

2010). It has been proposed that triazole PGRs interfere with cytochrome P₄₅₀ monooxygenases that convert ACC to ethylene (Grossman, 1992; Fletcher *et al.*, 2000). Alternatively, it has been suggested the catalyst is a dioxygenase with ascorbic acid as co-substrate and that acylcyclohexanedione PGRs, being structurally similar to ascorbic acid, inhibit dioxygenase activity (Rademacher, 2000; Rademacher *et al.*, 2006).

2.3.2.4 Cytokinin metabolism

Cytokinins stimulate cell division but are also involved in delay of senescence, lateral shoot development, chlorophyll biosynthesis and transpiration control (Hocart *et al.*, 1992; Fletcher *et al.*, 2000; Farooq *et al.*, 2009). The level of endogenous cytokinins has been increased with application of triazole (Grossman *et al.*, 1991; Fletcher *et al.*, 2000; Rademacher, 2000) and acylcyclohexanedione PGRs (Grossman *et al.*, 1994; Rademacher, 2000). The mechanism for this has not been elucidated but elevated levels are suggested to be associated with increased root growth, the site of major cytokinin activity (Fletcher *et al.*, 2000; Rademacher, 2000). Alternatively, inhibited ethylene biosynthesis may result in enhanced flux of SAM, which is also a precursor of cytokinins (Grossman, 1992). The isoprenoid cytokinin pathway is still largely unresolved (van Staden *et al.*, 2008), however it would appear possible that elevated levels of isoprenoid precursors through inhibition of GA biosynthesis would lead to increased cytokinin formation. Elevated chlorophyll biosynthesis has also been associated with PGR-induced increases in cytokinin levels (Grossman *et al.*, 1991). However, while chlorophyll content correlated well with rate of triazole, the association with cytokinin level was not close and Grossman *et al.* (1991) suggested that additional endogenous factors are involved in the stimulation of chlorophyll accumulation.

2.3.2.5 Flavonoid metabolism

Flavonoids are secondary metabolites with anti-oxidant properties produced by the plant as protection against various biotic and abiotic stresses (Pourcel *et al.*, 2007). In apples, Pro-Ca and TE have proven effective in reducing the incidence of fire blight, *Erwinia amylovora* (Rademacher, 2000) and apple scab, *Venturia inaequalis* (Roemmelt *et al.*, 2003; Costa *et al.*, 2004; Spinelli *et al.*, 2010). As these PGRs were found to exhibit little fungicidal or bactericidal effect (Roemmelt *et al.*, 2003), alternative modes of action were implied (Costa *et al.*, 2004; Rademacher *et al.*, 2006).

Flavonoid biosynthesis requires 2-oxoglutarate-dependent dioxygenase activity and, as with anabolism of other phyto-hormones, acylcyclohexanedione PGRs will inhibit this enzyme (Roemmelt *et al.*, 2003; Rademacher *et al.*, 2006). Resulting changes in the profile of flavonoids produce a new phenolic, luteoforal, which exhibits biocidal properties and, in particular, inhibits growth of *V. inaequalis* and *E. amylovora* (Rademacher *et al.*, 2006; Spinelli *et al.*, 2010). Reduced incidence of insect pests after Pro-Ca application is also possibly related to altered flavonoid composition (Rademacher *et al.*, 2006).

2.3.3 Effects of plant growth regulators on plant morphology and development

2.3.3.1 Stem characteristics

The major effect of anti-GA PGRs in cereal crops is a reduction in stem length, a direct result of reduced stem elongation through inhibition of active GA biosynthesis. In a detailed review of the literature, Berry *et al.* (2004) showed height reductions in cereals after PGR treatment ranged from no response to a 40% decrease. Further examination of previous studies shows that reductions are typically 10-20% at recommended rates and timing but there is considerable variation in PGR effect and these influences are expanded upon in Section 2.3.4. As discussed previously, reduced plant height through PGR activity results in a lower centre of gravity and decreased susceptibility to lodging. In cereals the peduncle and uppermost internodes being the longest, generally show the greatest reduction in elongation (Naylor, 1989; Tripathi *et al.*, 2003; Hussain and Leitch, 2007; Shekoofa and Emam, 2008). Shortened upper internodes after application of ETH in barley can also increase grain yield through reduced incidence of neck break at maturity (Batch, 1981).

A number of studies have also examined the effects of PGRs on plant vigour and height in poppies. Forbes and Laughlin (1985) in field trials screened several PGRs and found daminozide applied at the rosette stage was the most effective in reducing plant height. Application of ETH applied solely (Ramanathan, 1978; Hsu and Forman, 1982) and in combination with CCC (Nagy *et al.*, 1989), reduced plant vigour and stem height. PBZ, at high rates, reduced stem height in the latter study and also in ornamental poppy, *P. nudicaule* when applied as a soil drench (Martinetti *et al.*, 1994). In contrast, Srivastava and Sharma (1990) and Khan *et al.* (2007) recorded increases in plant height with the

PGR triacontanol. In these studies, effects on lodging were either not reported or there has been little provision of information due to work either being conducted in controlled conditions (Hsu and Forman, 1982; Srivastava and Sharma, 1990; Martinetti *et al.*, 1994; Khan *et al.*, 2007) or at field sites where conditions were not conducive to lodging (Nagy *et al.*, 1989). Only daminozide has been reported as effective in reducing lodging in poppies (Forbes and Laughlin, 1985), however toxicological concerns limit commercial application of this PGR in edible crops (Fletcher *et al.*, 2000; Rademacher, 2000).

In some studies with wheat, characteristics associated with stem strength have increased with application of PGRs, in particular stem diameter (Tolbert, 1960; Crook and Ennos, 1995; Tripathi *et al.*, 2003) and stem wall thickness (Tripathi *et al.*, 2003). Similarly in poppies, PBZ and a CCC/ETH mix applied at the start of bud development increased stem diameter (Nagy *et al.*, 1989). However, in other studies, there have been no effects from PGR application on these variables (Berry *et al.*, 2000; Matysiak, 2006). Perhaps of more value are direct mechanical tests to assess changes in stem strength. Application of Pro-Ca increased stem breaking force in rice (Kim *et al.*, 2007); however, with CCC applied to wheat, there was either no change (Crook and Ennos, 1995) or a decrease (Berry *et al.*, 2000) in stem strength and a slight reduction in stem rigidity (Crook and Ennos, 1995).

2.3.3.2 Root characteristics

The effect of PGR application on root growth has been examined in several field trials. Humphries *et al.* (1967) in one of the earliest studies reported that CCC-treated plants produced a greater mass of roots than control plants. In more detailed work, root density of wheat plants in the field was higher 3-4 months after application of CCC compared with untreated plants but by 6 months there was no difference (Bragg *et al.*, 1984). More recently, there were no significant effects on root length or biomass found with TE applied to barley in a field trial (Bingham and McCabe, 2006). Of note there was no yield benefit from the PGR treatment. Of these studies, only that of Humphries *et al.* (1967) showed an increase in grain yield.

Due to greater ease in separating root from soil, the effects of PGRs on root growth have been more commonly investigated in pot trials. Commercially recommended rates

of CCC, TE and ETH applied to wheat, barley and oats had no effect on root elongation (Rajala *et al.*, 2002). Although several greenhouse studies have shown an increase in the root: shoot ratio, this has been due to reduced shoot DM rather than increased root biomass in wheat (Rajala and Peltonen-Sainio, 2001) and barley (Rajala and Peltonen-Sainio, 2001; Emam and Shekoofa, 2009). The trials conducted by Rajala and Peltonen-Sainio concluded 14 days after application of TE but the higher root: shoot ratio was probably short term; in the study of Emam and Shekoofa, it was only after this time period that PGR effects on roots were detected and the root: shoot ratio subsequently decreased so that it was comparable with the untreated control. In this latter work, conducted in 90 cm soil columns, the effect of CCC on the roots was similar to above-ground responses with decreased elongation of the main root axis and increased branching of roots at shallow soil depths.

A number of technical difficulties in measuring root growth have been encountered in these studies. Large variability within treatments is common so that considerable differences in root biomass are not statistically significant (Rajala and Peltonen-Sainio, 2001), particularly at depth (Bragg *et al.*, 1984). In the field trial conducted by Bingham and McCabe (2006), overall depth of roots was relatively shallow (500 mm) for all treatments suggesting an impediment to root growth and, in the study by Humphries *et al.* (1967), root samples were extracted by pulling out plants, which is likely to be unreliable. In addition, many of the studies have been undertaken in controlled environment conditions and measurements conducted for an inadequate duration after PGR application (Rajala and Peltonen-Sainio, 2001; Woodward and Marshall, 1988). Pinthus (1973) pointed out that root DM is a poor indicator as it does not provide a measure of the spread of root mass, critical for lodging resistance. However, although number of coronal roots did increase in wheat with CCC application (Crook and Ennos, 1995), there was no beneficial effect on root anchorage strength (Crook and Ennos, 1995; Berry *et al.*, 2000).

Detailed information has been produced by some agricultural chemical manufacturers illustrating the benefit of PGR in improving root growth in cereals; however, there is a lack of consistent evidence in the scientific literature to support these claims and no subsequent yield increases have been reported. Work in other species has also been equally inconsistent with application of TE increasing root mass of turf grasses in some

studies (Baldwin *et al.*, 2006; McCullough *et al.*, 2006a) but no response documented in others (McCullough *et al.*, 2005; McCullough *et al.*, 2006b). More recently in canola, Berry and Spink (2009) have shown improvements in both scanned root length density and grain yield after application of MET. As will be discussed in Section 2.3.4, efficacy of PGR activity is influenced by a number of factors; however, it is clear greater research focus is required in this area.

While changes in total root biomass and structure in response to application of PGR in other studies are not clear, there is some evidence that the distribution of roots may be affected. In the study by Rajala and Peltonen-Sainio (2001), there was no significant effect on root growth of wheat but there was a tendency for root length and root area to increase in the 0-20 and 20-40 cm depth intervals and decrease at 40-60 cm. Similarly, Emam and Shekoofa (2009) recorded lower root DM below 45-60 cm after CCC application and this was also reflected in soil water uptake with less water extracted from the soil below 60 cm. As with root pruning (Fang *et al.*, 2010), this conserved water at depth may then be available later for grain-fill (Emam and Shekoofa, 2009) and through this mechanism, PGRs may allow greater tolerance to drought conditions.

2.3.3.3 *Maturity*

There are many reports in the literature of delayed plant development after application of PGRs and this has implications for the ensuing growing conditions. Time to ear emergence is generally not delayed for more than 2-3 days (Syme, 1968; Olumekun, 1996; Tripathi *et al.*, 2004; Toyota *et al.*, 2010) but up to 4-8 days delay has been observed in some studies in both wheat (Tolbert, 1960; Lowe and Carter, 1970) and barley (Foster and Taylor, 1993). Delay in inflorescence emergence after PGR treatment in wheat may be a function of the slower extension of the peduncle and spike within the leaf sheath (Olumekun, 1996) or a shorter peduncle length (Zagonel and Fernandez, 2007). Thus the delay may not be in plant reproductive development but an artifact of reduced stem extension. Several studies (Tripathi 2004; Toyota *et al.*, 2010) have recorded delays to anthesis, but only in the order of one day, and the fact that other studies have not observed any difference suggests this is a minor effect.

Perhaps of more importance, several studies have observed that application of PGRs, in particular CCC, has delayed senescence (Tolbert, 1960) and, in particular, of the flag

leaf (Naylor, 1989), peduncle and ear (Lowe and Carter, 1970). The latter study concluded that the delay in maturity and longer photosynthetic activity enhanced number of grains/ear however there was no corresponding increase in grain yield due to moisture stress during grain fill. Moes and Stobbe (1991) attributed a maturity delay in barley to later tillers that were initiated and formed ears after application of ETH. These additional later ears contributed to extra grain yield (Moes and Stobbe, 1991), but in other studies did not mature before autumn frosts (Foster *et al.*, 1991), or were adversely affected by drought conditions during grain-fill (Lowe and Carter, 1970).

2.3.4 Variability in effects of plant growth regulators

It has long been proposed that PGRs be used to modify and „fine tune’ cereal growth. However, in practice, apart from reducing stem height, PGRs have generally not lived up to expectations. Thus, while potential benefits of PGRs have been demonstrated, the previous discussion has also highlighted the large variation in plant responses. Response to PGR application is influenced by a number of factors and the action of a PGR is highly specific to a number of variables discussed below.

2.3.4.1 Plant species and variety

Across a number of studies, there have been large differences in specificity of different PGRs between species. In the first documented PGR research, Tolbert (1960) reported less effect of CCC on plant height of barley compared with wheat. This lack of height response was substantiated in subsequent work (Batch, 1981; Read, 1985; Rajala and Peltonen-Sainio, 2002) and also in reduced yield response (Mendham and Russell, 1985; Ma and Smith, 1991). For example, Rajala and Peltonen-Sainio (2002) found barley shoot growth largely unresponsive to CCC compared with wheat and oats whereas TE and ETH were effective in reducing plant height in all species. In rice, the triazole PGRs, PBZ and UNI (Hoffman, 1992) and acylcyclohexanedione PGRs, Pro-Ca (Kim *et al.*, 2007) and TE (Nascimento *et al.*, 2009) showed greater efficacy in reducing lodging than CCC or ETH. Similarly, PBZ and other triazole PGRs, MET and TEB have resulted in shorter plant stature, reduced lodging and higher grain yields in canola (Armstrong and Nicol, 1991; Baylis and Wright, 1990; Berry and Spink, 2009) but in the absence of disease had little effect on wheat or barley. Differences in response between varieties within a species have also been reported. Variety response to PGRs

have differed for grain yield, reduction in plant height and lodging in wheat (Wiersma *et al.*, 1986; Nafziger *et al.*, 1986) and barley (Moes and Stobbe, 1991) and effects did not appear to be related to plant height of control plants.

2.3.4.2 Crop stage of development

Early application of CCC, prior to and during tillering in wheat, has resulted in less effect on mature plant height compared with later applications (Syme, 1968; Perry and Miers, 1986). Similarly later applications of TE to wheat at early-mid stem elongation (GS32-33; Zadoks *et al.*, 1974) reduced stem height more than an early stem elongation (GS30-31) application (Zagonel and Fernandez, 2007; Espindula *et al.*, 2009). Comparable stem length reduction has also been noted with PGRs applied to barley (Waddington and Cartwright, 1986) and oats (Leitch and Hayes, 1990). It is likely that the reduced height response is due to lack of persistence of the PGR. Olumekun (1996) reported most effect on the first stem internode after CCC application (43% reduction) with progressively less response in subsequent internodes (37% and 15% respectively). In addition, efficacy of early PGR applications is reduced as this generally occurs when temperatures are lower (Berry *et al.*, 2004). Furthermore, later applications are generally applied at the time of elongation of the higher internodes and, as these are considerably longer than basal internodes (Batch, 1981; Pierre *et al.*, 2010), PGR effects are likely to be greater (Naylor, 1989; Tripathi *et al.*, 2003; Hussain and Leitch, 2007).

Given the potential influence on yield components from tillers and ears/m² to grains/ear and then grain weight, timing of the PGR should also progressively affect these yield components. There have been many studies where early application of PGRs has resulted in increased tiller population compared with later applications (Waddington and Cartwright, 1986; Ma and Smith, 1991; Rajala and Peltonen-Sainio, 2001; Hussain and Leitch, 2007). Similarly, application of CCC at mid-tillering in wheat increased final ears/m² whereas when applied at the start of stem elongation, grains/ear was increased (Hussain and Leitch, 2007). Timing of ETH application is particularly important as applications at or near flag leaf stage may reduce grains/ear due to ethephon-induced pollen sterility (Foster and Taylor, 1993).

2.3.4.3 Chemical uptake, activation and duration of activity

Differences in the chemical nature and mode of action of PGRs result in variation in response to application. Uptake sites vary; while most PGRs are preferentially absorbed through leaves and stems (Fagerness and Penner, 1998; Kim *et al.*, 2010), UNI and PBZ are less well absorbed by leaves and show greater efficacy applied to horticultural trees through the stem (Fletcher *et al.*, 2000) or roots as a soil drench (Rademacher, 2000). Metabolic activation and duration of activity also differ between PGRs with Pro-Ca being faster acting with a low residual (Rademacher *et al.*, 2006; Kim *et al.*, 2007; Kang *et al.*, 2010) whereas activity of CCC and MEQ is more delayed and persistent (Rademacher, 2008). Some triazoles such as UNI and PBZ have a half life in both plant and soil of several months (Rademacher, 2000) whereas others such as TEB and MET are less persistent and Fletcher *et al.* (2000) considers there is a relationship between efficacy and persistence. There may also be differences between plant types; TE is rapidly absorbed but activation requires conversion to an acidic form that occurs readily in monocotyledonous plants but not in many dicotyledons (Rademacher, 2000; Spinelli *et al.*, 2010).

2.3.4.4 Rate of PGR applied

High rates of applied PGR can affect crop yield. Twice the recommended rate of ETH significantly decreased the number of grains/ear and increased sterile tillers compared with the recommended rate in barley (Foster *et al.*, 1991) and decreased yield in wheat (Nafziger *et al.*, 1986). Similarly, TE applied at rates only 50% higher than recommended, reduced grain yield in wheat (Zagonel and Fernandez, 2007; Espindula *et al.*, 2009). Higher rates tended to reduce leaf area and with a lower total biomass, there was less stem water-soluble carbohydrate available for grain-fill (Espindula *et al.*, 2009). Zagonel and Fernandez (2007) also observed that at higher rates of TE, the peduncle length was shortened so that not all ears emerged fully from the flag leaf sheath, resulting in reduced grain yield. Leitch and Hayes (1990) suggested that as the mode of action of PGRs is to retard plant growth, higher rates may reduce vegetative structure to a level where yield is impacted.

Greater reduction in plant height with higher PGR rates is also commonly recorded. In the studies of Zagonel and Fernandez (2007) and Espindula *et al.* (2009), there was a linear reduction in wheat plant height with increasing rate of TE; comparable results

have been documented by Matysiak (2006). Similar incremental decreases in crop height with increasing rates of other PGRs have also been reported with CCC (Perry and Miers, 1986) and ETH in wheat (Nafziger *et al.*, 1986) and barley (Foster *et al.*, 1991).

2.3.4.5 *Weather and growing conditions*

As with other agricultural chemicals, weather conditions at the time of application will influence efficacy and, if unfavourable, may lead to side effects and damage to the crop. Release of ethylene is likely to be enhanced with elevated temperatures following application of ETH and Moes and Stobbe (1991) proposed that this reduced grains/ear in barley. Poole (2005) cites a range of 15-20°C for product efficacy of ETH. Optimal temperature for different PGRs varies but it is generally above 5°C (Berry *et al.*, 2004).

Environmental and growing conditions subsequent to PGR application will also have a large impact on the response and add another level of complexity. In more favourable seasons, greater height reductions in response to PGR application have been reported in multi-year studies with CCC (Foster and Taylor, 1993; Rajala and Peltonen-Sainio., 2002), TE (Rajala and Peltonen-Sainio., 2002; Matysiak, 2006) and ETH (Rajala and Peltonen-Sainio., 2002). Examination of the data in a study by Foster *et al.* (1991) shows expression of higher tiller numbers after ETH application only occurred in a season of above average rainfall. Similarly, higher grain yield potential after application of TE was realized through increased grains/ear in a season with high rainfall but there was no yield response in a year of average rainfall (Matysiak, 2006). Dry conditions after application of ETH can even result in yield losses in barley (Knapp *et al.*, 1987) and Poole (2005) suggests 5-8% yield losses are common.

2.3.4.6 *Crop management*

Crop growth and thus response to PGRs is also altered by different management inputs. A number of studies have investigated the effect of PGR application with varying nitrogen status. At high rates of nitrogen, application of CCC to wheat increased grain yield (Syme, 1968) and, in other studies, this was through increased grains/ear (Lowe and Carter, 1970) or ear numbers (Shekoofa and Emam, 2008). Naylor (1989) also reported relatively low incidence of lodging with CCC application at nitrogen rates of 180 kg/ha compared with untreated plots. While the trend was towards greater yield with high nitrogen coupled with CCC, this effect was not significant. Of note however,

this trial was hand harvested and likely to result in higher yields for lodged plots compared with mechanical harvest.

Differences in sowing rate and time may also influence response to PGR treatment. Grain yield in barley at densities of 250 and 500 plants/m² tended to increase after application of CCC and MEQ. In contrast, at 100 plants/m² both PGR treatments tended to decrease yield and this was close to statistical significance (Waddington and Cartwright, 1986). Application of MEQ at a normal sowing date increased grain yield of a barley variety whereas with a one month delay in sowing, yield tended to decrease (Waddington and Cartwright, 1986). Similarly, in a comparison of autumn and spring sown oats, panicle numbers were higher for the early sowing and increased with application of CCC. In contrast, CCC had no effect on this yield component with a spring sowing (Browne *et al.*, 2006).

2.3.5 The role of canopy size in PGR function

As discussed, increased grain yield with application of PGRs under conditions conducive to lodging has been commonly reported and many authors have concluded that application of PGRs is unlikely to be economic unless growing conditions result in at least moderate levels of lodging and thus grain reduction (e.g. Rajala and Peltonen-Sainio, 2002; White *et al.*, 2003; Tripathi *et al.*, 2004). However several authors have reported a poor correlation between grain yield and lodging (Foster and Taylor, 1991; White *et al.*, 2003; Tripathi *et al.*, 2004) and Berry *et al.* (2004), in a review of the effects of PGRs on cereals, documented that in the absence of lodging, across 60 studies there were yield increases in only 13. Furthermore, as discussed Section 2.2.3.4, a large number of agronomic variables affect severity and susceptibility to lodging. For example, while lodging may be severe, late lodging has less effect on grain yield and consequently although PGRs may reduce lodging, this may be of little yield benefit (Pinthus, 1973; Fischer and Stapper, 1987; Tripathi *et al.*, 2003). Thus the use of site lodging potential as a guide to PGR benefit or otherwise is flawed.

Larger canopy size is associated with higher crop inputs and favourable growing conditions and the beneficial effects of PGR application are often only expressed in interactions with other management inputs or environmental conditions. For example, in

PGR x nitrogen studies, high rates of nitrogen coupled with PGR application increased grain yield compared with other treatment combinations of lower nitrogen or absence of PGR (Syme, 1968; Lowe and Carter, 1970; Naylor, 1989; Shekoofa and Emam, 2008). Other crop management inputs such as irrigation (Read, 1985), higher sowing rates (Waddington and Cartwright, 1986) and earlier time of sowing (Waddington and Cartwright, 1986; Browne *et al.*, 2006) have also resulted in greater yield response to PGR treatment.

Under conditions of favourable growth, crop canopy development will generally be optimal and in many cases supra-optimal. It is under these latter conditions of excess vegetative growth that in addition to increased incidence of disease, crop efficiency will be reduced as measured by water and radiation use efficiency and harvest index. With high management inputs and denser tiller stands, stems may be more elongated (Pinthus, 1973) with higher centre of gravity (Berry *et al.*, 2000) and reduced stem and anchorage strength (Crook and Ennos, 1995). While increased lodging can be a major feature and yield determinant under such growing conditions, it is generally only symptomatic of excess canopy size and, as such, a poorer indicator of the benefits of PGR use than direct canopy measurements such as leaf or green area index. Berry and Spink (2009) have recently demonstrated this with a series of trials in canola in which variation in grain yield was largely explained by canopy size at the time of application of MET. It is thus canopy size that should provide a more accurate measure of the potential for a crop to benefit from application of a PGR.

By reducing excess above-ground biomass, PGRs may assist with partitioning of resources into either storage organs and/or additional yield components. PGRs may also directly improve canopy architecture and increase crop growth and yield efficiency. In wheat, application of TE improved canopy architecture by producing more erect leaves (Espindula *et al.*, 2009) and more recently, CCC applied at anthesis, increased the mean tip angle, a measure of leaf orientation (Toyota *et al.*, 2010). More erect leaves, particularly in the upper canopy will reduce shading of lower leaves and provide more efficient utilization of radiation within the canopy leading to greater photosynthetic performance (Yunasa *et al.*, 1993; Espindula *et al.*, 2009). However, as surmised by Berry and Spink (2009), application of PGRs may also reduce leaf area below an optimal size. Thus where canopy development is less than supra-optimal a reduction in

plant growth is likely to decrease radiation absorption and photosynthetic efficiency and depending on canopy size, may reduce yield (Berry and Spink, 2009).

The association between potential PGR efficacy and canopy size provides an alternative explanation for response to PGRs. For example, under high input conditions, tall varieties with a large biomass are likely to produce luxuriant canopy growth and consequently respond beneficially to PGR application even in the absence of lodging. In contrast, canopy size of a shorter variety, growing under the same conditions, may not have exceeded a critical value. Under less favourable growing conditions, application of PGR on the latter may result in a sub-optimal canopy and thus reduce yields.

If the role of PGRs is re-appraised from that of reducing lodging to one of controlling or checking excess vegetative growth, the hormonal role of PGRs should also be reconsidered. As discussed in Section 2.3.4, responses to PGRs are expressed through altered hormonal balance with decreases in GA (e.g. Rademacher, 2000; Kim *et al.*, 2007; Kang *et al.*, 2010) leading to reduced stem elongation. In addition, application of triazole and acylcyclohexanedione PGRs has also increased levels of ABA (Hauser *et al.*, 1990; Grossman, 1992; Chizhova *et al.*, 2005; Bingham and McCabe, 2006) and associated with this, decreases in stomatal opening, respiration and transpiration rates and water use (Hauser *et al.*, 1990; Grossman, 1992; Fletcher *et al.*, 2000). These effects lead to a check in growth and reduced dry matter accumulation. Under conditions where canopy development is less than supra-optimal, PGR application, by inducing a stress response, is likely to decrease grain yield. However, with more extreme levels of biotic or abiotic stress, there may again be benefits with PGR application stemming from reduced growth. As discussed in Section 2.3.2, increased levels of ABA following application of PGRs have been implicated in improved tolerance to cold, heat and herbicide damage (Grossman, 1992; Gilley and Fletcher, 1997; Fletcher *et al.*, 2000). While this increased stress tolerance may be a result of improved anti-oxidant activity (Fletcher *et al.*, 2000; Jaleel *et al.*, 2006), the response may also be adaptive with reduced plant growth ensuring that canopy size is more in balance with the unfavourable environmental conditions.

2.3.6 Summary and scope of thesis

From this literature review it is clear that PGRs are used on a broad range of crops to modify vegetative growth and in cereals, in particular, successfully reduce crop lodging. The aim of this research was to screen a range of these PGRs and growth suppressants for effectiveness in reducing the incidence of lodging in poppies. However, results from other crops have often been variable and this is not surprising given the complex interactions that occur between plant hormones and their response to the environment that, in turn, is influenced by factors such as weather conditions, crop management, growth stage at time of PGR application and rates applied. Thus, initial evaluation in this study required a broad range of PGRs applied at different rates and growth stages across several seasons. There is also some indication that yield components and quality can be affected by PGR application. Given the high value of poppy crops, the potential of PGRs to at least maintain crop yield and alkaloid content is essential and was also assessed. In particular production of high alkaloid is cost effective for industry and ensures higher returns for growers. Thebaine is also of higher value than oripavine and effects on alkaloid profile in particular were monitored.

CHAPTER 3. SCREENING OF PLANT GROWTH REGULATORS TO REDUCE LODGING IN POPPIES

3.1 Introduction

Plant growth regulators and suppressants are applied in a number of agricultural and horticultural crops to reduce vegetative growth and potentially modify yield and its components. There are two main categories of PGRs, inhibitors of GA biosynthesis and ethylene producing compounds (Grossman, 1992; Rademacher, 2000) and their modes of action are detailed in Chapter 2. Most commercial PGRs inhibit GA formation with different classes of anti-GA PGRs blocking activity of specific enzymes. Cycocel and Pix (see Table 2.1 for common chemical names) are commonly used as PGRs in cereals and inhibit steps early in GA biosynthesis. The triazole PGRs including Cultar, Sunny, Caramba and Folicur are registered for a range of crops from horticultural trees to canola (Fletcher *et al.*, 2000; Dapprich *et al.*, 2002; Rademacher and Brahm, 2010) and inactivate monooxygenase enzymes involved in a series of oxidation steps (Grossman, 1992; Rademacher, 2000). Triazoles commonly occur as different enantiomers with varying activity on GA and sterol biosynthesis (Fletcher *et al.*, 2000; Rademacher, 2000). Inhibition of the latter is implicated with fungicidal properties. While Folicur and Opus are primarily recognised as fungicides, they also reduce GA production in canola (Kightley, 2001; Dapprich *et al.*, 2002) and cleavers (*Galium aparine*) (Benton and Cobb, 1995) respectively. Acylcyclohexanedione PGRs such as Moddus and Apogee also inactivate oxidation steps but through blocking of dioxygenase enzymes later in the GA pathway (Rademacher, 2000) and are applied to cereals, grasses and pome fruit (Rademacher and Brahm, 2010). Ethephon based products such as Ethrel have a different mode of action and degrade to form ethylene (Yang, 1969; Moshkov *et al.*, 2008), which at enhanced levels inhibits cell elongation (Rajala *et al.*, 2002). Ethephon is used for crop thinning and/or to accelerate ripening in cotton and a range of horticultural fruit crops but is also applied to barley to reduce plant height and the potential for lodging (Foster *et al.*, 1991; Rademacher and Brahm, 2010).

In poppies (*Papaver spp.*), plant vigour and height have been reduced with application of paclobutrazol (Nagy *et al.*, 1989; Martinetti *et al.*, 1994), daminozide (Forbes and

Laughlin, 1985) and Ethrel applied solely (Ramanathan, 1978; Hsu and Forman, 1982) and in combination with chlormequat (Nagy *et al.*, 1989). Reduced lodging was reported with daminozide applied at the rosette stage in a field trial (Forbes and Laughlin, 1985); however toxicological concerns limit commercial application of this PGR in edible crops (Fletcher *et al.*, 2000; Rademacher, 2000). Effects on lodging have not been reported in other studies due to either being conducted in controlled conditions (Hsu and Forman, 1982; Srivastava and Sharma, 1990; Martinetti *et al.*, 1994; Khan *et al.*, 2007) or at field sites where conditions were not conducive to lodging (Nagy *et al.*, 1989). More recently an initial screening of PGRs in morphine poppies for effects on plant height and lodging and subsequent responses in yield and alkaloid content was conducted by Dean and Fist in 2001-02 (unpublished). Responses ranged from no visible effects on plant height to large reductions with Ethrel and Apogee applied at early and mid stem elongation. Incidence of lodging was also reduced by high rates of Ethrel and tended to decrease with Apogee and Moddus treatments. Ethrel also reduced capsule size and yield largely through a decrease in seed yield and alkaloid content. Application of Cycocel and Cultar had little effect on growth or yield parameters. However, the acylcyclohexanedione PGRs, Apogee and in particular Moddus, both altered alkaloid profile with a greater proportion of thebaine and less morphine and Moddus also tended to decrease seed and increase straw yield. The success of these trials demonstrated the potential for PGRs to not only decrease plant height in poppies but importantly, to also modify yield and alkaloid parameters. Researchers with GlaxoSmithKline in Tasmania also found treatment with the triazole PGR, Sunny effectively decreased plant height and showed the potential to increase crop yield (Sumitomo, 2010).

Other chemicals such as herbicides applied at lower than recommended rates can also reduce plant growth without causing evident phytotoxicity (Pinthus, 1973; Rademacher and Brahm, 2010). The herbicides, Eclipse (metosulam) and Reglone (diquat), are used commercially in poppy crops. Reglone is generally non-selective but due to leaf surface characteristics causes only minor damage when applied to poppies (Fist, 2001). While both herbicides reduced plant height when applied at the rosette stage prior to stem elongation, effects were temporary and masked by rapid growth during stem elongation (Dean, unpublished). Of note, however, higher rates of Eclipse tended to increase straw yield. Eclipse can be applied until the onset of stem elongation but lower rates must be

used compared with more timely applications (P. Lindsay, pers. comm.). Slow Grow (maleic hydrazide) is also a growth suppressant, predominantly applied to control premature sprouting of onions and potatoes (Fletcher *et al.*, 2000, Rademacher and Brahm, 2010) but also has herbicidal activity on some broad leaf weeds (Fletcher *et al.*, 2000). It is therefore of merit to not only evaluate PGRs but in addition some herbicides with potential to reduce poppy growth.

The aim of this research was to screen a range of PGRs for effectiveness in reducing the incidence of lodging and potentially increasing alkaloid and crop yield in poppies. The herbicide, Eclipse and growth suppressant, Slow Grow were also trialled. Selected treatments were applied at different growth stages and in combinations to determine if PGR effects were cumulative. With the increased thebaine levels after application of Moddus in the initial screening being of significant economic benefit, PGRs in this study were applied to thebaine poppies.

3.2 Materials and methods

3.2.1 Field trial design and treatments

Three screening trials were conducted in northern Tasmania, one preliminary and two fully replicated, to examine the effect of different PGRs on growth, yield and alkaloid content of thebaine poppies. A list of PGRs used in these trials is presented in Table 2.1 and trial design and treatment information in Table 3.1. In the preliminary screening trial (Cressy, 2002-03), the potential of each PGR was assessed with three rates (x, 2x and 4x) and all PGRs were applied at early stem elongation as the main stem emerged from the plant rosette (see Figure 2.1 for diagram of growth stages). With the large number of treatments, full replication was not manageable and an incomplete block design was used containing a spatial grid of standard (control) plots at intervals of 4-5 plots. PGRs showing the greatest potential were further evaluated in replicated screening trials at Symmons Plains (Symmons) in 2003-04 and Hagley, 2004-05. Only check plots of Moddus and Sunny were included in the latter screening trials as these PGRs were investigated in further trials (Chapters 4 and 5). Additional treatments at

Hagley, 2004-05, were applied at mid to late stem elongation (Table 3.1) and included some combinations of PGR treatments (Table 3.4) to determine if effects were additive.

Table 3.1. Site and trial information for PGR field trials, 2002-2004.

Year	2002-03	2003-04	2004-05
Site	Cressy	Symmons	Hagley
Latitude / longitude	41° 45'S; 147° 03'E	41° 38'S; 147° 15'E	41° 30'S; 146° 53'E
Soil type	sodosol	sodosol	dermosol
Previous crop	pasture	lupins	onions
Trial and design	Preliminary	Replicated	Replicated
	1-2 replicates	2- 3 replicates	2- 3 replicates
	Spatial grid	Incomplete block	Incomplete block
Applications	single	single	single, mixes
Growth stages	eSE	eSE	eSE; emSE; mlSE
Sowing date	3 Oct 2002	9 Sep 2003	18 Aug 2004
rate (g/ha)	800	800	800
Basal N:P:K fertiliser	9-14-17	10-18-8 + B	10:17:8
rate (kg/ha)	300	400	400
Harvest date	3 Feb 2003	9 Feb 2004	25 Jan 2005
area/plot	15.2 m ²	16.2 m ²	15.2 m ²

SE, stem elongation; e = early; em, early to mid; ml, mid to late.

3.2.2 Cultural details

Trials were sown in commercial poppy paddocks and crop management followed paddock operations. Details of trial site soil type and paddock history are provided in Table 3.1. Weeds and diseases were controlled by application of commercially registered herbicides and fungicides at appropriate stages of crop growth. Irrigation and top-dressed fertiliser were applied in line with commercial practice. Trial PGR treatments were applied using a knapsack sprayer fitted with Hardi no. 10 nozzles. The knapsack was operated at approximately 250 kPa pressure with a walking speed of close to 1 m/sec. Additives were applied as per PGR label directions, generally being BS 1000. As recommended, ammonium sulphate was added during all tank mixing of

Apogee treatments as in a preliminary trial there tended to be increases in straw and seed yield compared to no additive (data not presented).

3.2.3 Trial measurements and harvest procedure

After application of PGRs, visual effects of treatments and incidence of lodging were recorded and, prior to harvest mature, plant height was measured. Poppies differ from most crops as the most economically important component of the plant is the capsule that at maturity, is the major site of alkaloid deposition (Chung, 1987; Srivastava and Sharma, 1990). As crop payment structure favours high alkaloid content (Fist, 2001), the amount of stem harvested mechanically with the capsule is minimised (Laughlin, 1987). Capsules were hand harvested at maturity by snapping individual capsules off at the abscission zone directly below the receptacle. Capsules from the shortest and most immature plants were left as commercially these would not be harvested.

3.2.4 Sample analysis

Harvested samples were weighed before seed removal (capsule yield) and individual capsule weight determined from the mean of 30 capsules. The term “straw” in the poppy industry refers to the harvested capsule walls or pericarp and the small section of stem immediately below the capsule. Straw yield was measured after removal of seed by threshing. With the majority of alkaloid concentrated in the capsule wall and nil or negligible levels in the seed (Khan *et al.*, 2007; Mahdavi-Damghani *et al.*, 2010), the ratio of straw/capsule (S/C) is an important yield parameter (Chung, 1987) and is a measure of poppy yield harvest index.

Straw samples were ground and a 2 g sub-sample extracted with 50 ml of 5% ethanol acidified with 0.18% v/v phosphoric acid for 60 minutes. Alkaloids were then analysed by reverse phase HPLC against pure standards (A. Fist, pers. comm.). Alkaloid content was expressed as a percentage of the straw yield. The total alkaloid content was the sum of alkaloids of which morphine and codeine are the most predominant in morphine crops and thebaine and oripavine in thebaine poppy crops. The thebaine ratio (T ratio) provides a measure of the alkaloid profile and is the proportion of thebaine content to total alkaloid content. A high T ratio is desirable for the industry as oripavine is of

lower value than thebaine. Similarly, the morphine ratio (M ratio) used in this study is the ratio of morphine content to the total of morphine plus codeine content. Alkaloid yield is the straw yield multiplied by alkaloid content.

3.2.5 Statistical analysis

For the preliminary trial (Cressy, 2002-03), a residual maximum likelihood (REML) regular grid spatial model was used to predict treatment means. This model is able to correct for two dimensional site variations and is particularly effective with limited replication. In determining statistical significance, an unbalanced treatment structure was used. For replicated trials (Symmons 2003-04, Hagley 2004-05), an unbalanced ANOVA was used with means predicted from a general linear regression model. Data were analysed using Genstat version 12.1. Due to insufficient replicates, an analysis of interaction between rate and time of application was not conducted. As the number of replicates in each trial varied, least significant differences (l.s.d.) for testing differences between means at the 5% level were calculated from the unbalanced model.

3.3 Results

Monthly rainfall and temperature data is presented in Appendix 1. The closest long term weather stations to the Symmons and Hagley sites were Launceston and Westbury (rainfall only), 12 and 4 km distant, respectively. Rainfall with supplemental irrigation at the Cressy and Hagley sites was sufficient to enable optimal establishment. To avoid potential water-logging, poppies at Symmons were sown on raised beds and with slight variation in the number of rows on top of beds, plot establishment tended to be more variable. Subsequent early growth at the Symmons site was reduced by below average rainfall until irrigation commenced in late October. Crop growth at Hagley was particularly high due to a well-structured, fertile soil type. After application of PGRs maximum daily temperature in 2002-03 at the Cressy site remained above 25°C for a week and was as high as 29°C. Of note, at Symmons there was 109 mm of rainfall over the 2 week period prior to harvest.

3.3.1 Preliminary PGR screening trial: Cressy 2002-03

3.3.1.1 The effect of PGR application on plant growth and yield components

A number of PGRs produced significant effects on growth, in particular height (Table 3.2). High rates of Eclipse, Apogee, Ethrel and Moddus reduced plant height compared with control plots with the 2 g and 4 g/ha applications of Eclipse being most effective. Ethrel and Eclipse also tended to produce smaller capsules (Table 3.2) and those of Ethrel treated plants were still immature at harvest. Slow Grow applied at 10 and 20 L/ha also reduced plant height (means of 76.6 and 67.9 cm respectively) however capsules were very small and hence were not harvested. Lower rates of Slow Grow did not significantly reduce plant height but capsules still tended to be smaller.

There were significant PGR treatment effects on capsule and seed yield (Table 3.2). Slow Grow applied at 5 L/ha and the higher rates of Moddus, Ethrel and Eclipse reduced capsule yields largely due to significant decreases in seed yield compared with the control. Straw yield did not change and this was reflected in significantly higher S/C ratios for these PGRs. Conversely, higher rates of Apogee tended to increase seed weights with a correspondingly lower S/C ratio.

3.3.1.2 The effect of PGR application on alkaloid

Application of 5 L/ha of Slow Grow, all rates of Moddus and the high rates of Ethrel and Eclipse increased thebaine level (Table 3.2). For Moddus and to a lesser degree Eclipse, this was associated with reduced oripavine content. The effect of Moddus was particularly marked with an average 54% reduction in oripavine and this was rate responsive. Application of the other acylcyclohexanedione PGR, Apogee, also decreased oripavine. In contrast, treatment with Slow Grow (5 L/ha) and the highest rates of Ethrel (1.2 L/ha) tended to increase oripavine content thus resulting in higher total alkaloid content compared with the control. Differences in total oripavine yield largely reflected the differences in oripavine content.

Table 3.2. Effect of different PGR treatments on growth, yield and alkaloid parameters in the preliminary field trial at Cressy, 2002-03.

Data are predicted means. All treatments applied at early stem elongation.

Treatment (/ha)	no. reps	Plant ht (cm)	Capsule wt wt (g)	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Tot alk yld (g/m ²)
Control	8	83.8	4.51	239.6	96.9	140.8	0.404	1.999	0.815	0.715	2.812	1.939	0.771	2.683
0.75kg Apogee	2	79.3	4.79	235.3	96.8	137.7	0.409	2.005	0.729	0.737	2.723	1.913	0.673	2.569
1.5kg Apogee	3	77.0	4.17	241.0	95.7	148.6	0.399	2.031	0.655	0.764	2.681	2.004	0.613	2.563
3.0kg Apogee	1	66.4	4.21	241.4	95.1	152.6	0.391	2.153	0.596	0.784	2.742	2.076	0.567	2.602
0.2L Cultar	1	86.0	4.92	241.5	98.6	131.4	0.410	1.892	0.776	0.711	2.663	1.885	0.738	2.507
0.4L Cultar	2	81.8	5.00	253.0	100.5	132.2	0.402	2.105	0.832	0.717	2.933	2.026	0.781	2.721
0.8L Cultar	2	80.8	4.67	249.8	94.9	147.3	0.392	1.997	0.820	0.712	2.816	1.938	0.791	2.689
1.0g Eclipse	1	70.6	4.06	196.8	96.4	95.0	0.494	2.059	0.758	0.737	2.809	1.992	0.696	2.610
2.0g Eclipse	1	56.6	4.16	203.3	102.4	95.2	0.499	2.108	0.679	0.764	2.778	2.117	0.614	2.583
4.0g Eclipse	1	51.6	2.99	160.6	97.8	54.5	0.605	2.288	0.576	0.804	2.853	2.239	0.520	2.673
0.3L Ethrel	2	81.5	3.87	214.1	88.3	124.2	0.405	1.983	0.744	0.721	2.734	1.712	0.652	2.363
0.6L Ethrel	1	76.4	3.37	196.2	91.3	99.2	0.470	2.227	0.828	0.725	3.063	2.013	0.777	2.814
1.2L Ethrel	1	69.4	3.61	166.9	93.6	56.9	0.581	2.469	0.904	0.727	3.371	2.144	0.806	2.963
0.75L Moddus	2	82.5	4.35	209.0	87.6	122.2	0.418	2.291	0.485	0.822	2.778	2.023	0.429	2.436
1.5L Moddus	2	80.5	3.65	190.9	81.3	109.8	0.426	2.397	0.358	0.866	2.758	1.951	0.295	2.237
3.0L Moddus	2	76.5	4.13	193.3	88.5	109.3	0.449	2.426	0.278	0.891	2.710	2.124	0.267	2.376
0.25L Opus	1	81.0	4.02	177.5	75.0	109.9	0.428	1.932	0.764	0.721	2.704	1.488	0.585	2.156
0.5L Opus	1	82.0	4.15	198.0	80.0	134.7	0.405	2.009	0.797	0.717	2.807	1.594	0.663	2.457
1.0L Opus	1	82.0	5.52	209.3	87.1	130.8	0.417	2.074	0.866	0.709	2.940	1.818	0.759	2.692
0.25L Pix	2	84.2	4.63	231.5	95.9	148.2	0.403	2.041	0.768	0.730	2.801	2.022	0.745	2.772
0.5L Pix	2	84.0	4.22	240.7	100.9	143.1	0.414	2.089	0.782	0.732	2.872	2.109	0.771	2.853
1.0L Pix	2	84.7	4.69	211.3	92.6	131.9	0.424	2.059	0.763	0.731	2.815	1.959	0.716	2.674
5.0L Slow Grow	1	83.0	3.38	152.4	84.8	76.0	0.558	2.533	0.946	0.739	3.484	2.230	0.804	3.047
<i>F prob</i>		<.001	0.179	<.001	0.345	<.001	<.001	<.001	<.001	<.001	0.040	0.223	<.001	0.056
<i>l.s.d. 0.05</i>		5.9	<i>n.s.</i>	41.2	<i>n.s.</i>	26.1	0.032	0.247	0.135	0.026	0.339	<i>n.s.</i>	0.136	0.525

3.3.2 PGR screening trial: Symmons, 2003-04

Plant height was reduced with higher rates of Sunny (Table 3.3) and tended to be lower with all rates of Apogee. Sunny treatments were observed to delay maturity and increase the number of lateral stems (data not shown). Application of Moddus decreased oripavine and tended to increase thebaine content resulting in a higher T ratio than other PGRs and the control (Table 3.3). In contrast, higher rates of the other triazole PGRs, Sunny and Folicur tended to increase oripavine content.

3.3.3 PGR screening trial: Hagley, 2004-05

3.3.3.1 *The effect of PGR application on plant growth and yield components*

Plant height was significantly decreased with application of Sunny and the higher rate of Apogee and tended to be reduced with Caramba and Slow Grow compared with control plots (Table 3.4). Minor lodging occurred in two of the control plots with none in the PGR treatments. Straw yields were not significantly different ($P = 0.09$) between treatments. However, there were some interesting trends. For example, application of Slow Grow at early stem elongation and most Moddus treatments, applied both solely and in combination with Slow Grow, tended to increase straw and decrease seed yield (Table 3.4). In contrast, application of Sunny tended to increase seed yield thereby significantly lowering the S/C ratio. The high rate of Apogee also tended to increase seed yield and when applied at mid-late stem elongation in combination with Moddus, the S/C ratio was significantly lower than that of Moddus alone.

3.3.3.2 *The effect of PGR application on alkaloid*

All Moddus and Moddus combinations significantly reduced oripavine content and tended to increase thebaine content ($P = 0.08$) and consequently there was little difference in total alkaloid content between Moddus and control treatments (Table 3.4). Slow Grow applied at early stem elongation also tended to reduce oripavine content and significantly increased the T ratio. In contrast, application of Sunny increased oripavine content resulting in a lower T ratio compared with the control treatment. Similar effects were also expressed in alkaloid yield data with most Moddus and Moddus combinations producing higher thebaine and lower oripavine yields than control treatments.

Table 3.3. Effect of different PGR treatments on growth, yield and alkaloid parameters at Symmons Plains, 2003-04.

Data are predicted means. All treatments applied at early stem elongation.

Treatment (/ha)	Plant ht (cm)	Capsule wt wt (g)	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Tot alk yld (g/m ²)
Control	87.0	4.17	203.3	68.6	134.7	0.337	1.900	0.645	0.741	2.544	1.308	0.447	1.754
1.0kg Apogee	81.1	4.04	163.5	56.9	106.5	0.350	1.891	0.544	0.753	2.435	1.072	0.315	1.387
2.0kg Apogee	78.8	3.82	201.0	70.3	130.6	0.351	1.893	0.538	0.761	2.431	1.331	0.385	1.717
4.0kg Apogee	77.6	3.54	199.4	67.1	130.9	0.344	1.785	0.578	0.759	2.362	1.201	0.389	1.590
0.75L Caramba	82.1	3.73	168.6	58.8	109.7	0.348	1.873	0.501	0.788	2.374	1.102	0.291	1.393
0.75g Eclipse	85.1	4.29	230.6	77.5	153.1	0.337	2.043	0.704	0.744	2.747	1.559	0.544	2.103
1.5g Eclipse	87.8	3.88	216.2	74.2	142.0	0.345	1.875	0.621	0.750	2.496	1.371	0.458	1.829
3.0g Eclipse	84.8	4.15	217.1	73.7	143.4	0.339	1.890	0.664	0.712	2.553	1.380	0.494	1.874
0.6L Folicur	84.8	4.11	203.3	71.5	131.8	0.354	2.037	0.682	0.751	2.720	1.448	0.496	1.944
1.2L Folicur	90.1	4.38	227.3	76.7	150.6	0.337	1.869	0.695	0.730	2.564	1.443	0.540	1.983
2.0L Moddus	84.5	4.04	181.2	67.8	113.4	0.374	2.100	0.416	0.839	2.517	1.466	0.299	1.764
1.5L Slow Grow	84.7	3.87	214.4	71.0	143.4	0.330	1.711	0.571	0.751	2.282	1.219	0.411	1.630
3.0L Slow Grow	82.4	4.14	191.5	68.5	123.1	0.359	1.999	0.660	0.750	2.659	1.364	0.450	1.814
0.75L Sunny	76.8	3.32	218.0	74.1	143.9	0.340	1.840	0.562	0.766	2.402	1.360	0.415	1.774
1.5L Sunny	65.8	2.95	195.7	66.8	128.9	0.342	1.927	0.740	0.727	2.667	1.286	0.496	1.782
3.0L Sunny	63.5	3.71	195.8	65.8	130.1	0.341	1.946	0.699	0.736	2.645	1.276	0.458	1.734
<i>F prob</i>	<i><.001</i>	<i>0.362</i>	<i>0.412</i>	<i>0.621</i>	<i>0.309</i>	<i>0.212</i>	<i>0.519</i>	<i>0.041</i>	<i>0.001</i>	<i>0.466</i>	<i>0.626</i>	<i>0.273</i>	<i>0.624</i>
<i>l.s.d. 0.05</i>	<i>11.6</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.180</i>	<i>0.046</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Table 3.4. Effect of different PGR treatments on growth, yield and alkaloid parameters at Hagley, 2004-05.

Data are predicted means. SE, stem elongation; e, early; em, early-mid; ml, mid to late; M, Moddus.

Treatment (/ha)	Plant ht (cm)	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Tot alk yld (g/m ²)
Control	124.9	343.1	133.3	209.8	0.389	2.566	0.930	0.735	3.496	3.401	1.238	4.639
1.5kg Apogee eSE	121.7	351.7	138.2	213.5	0.393	2.576	0.902	0.740	3.478	3.555	1.251	4.807
3.0kg Apogee eSE	117.8	388.7	149.1	239.7	0.384	2.753	0.868	0.760	3.621	4.107	1.294	5.401
3.3L Caramba eSE	120.2	384.4	150.9	233.5	0.394	2.498	0.976	0.718	3.474	3.763	1.476	5.239
1.0g Eclipse eSE	122.2	381.1	147.3	233.7	0.387	2.797	1.031	0.730	3.829	4.117	1.522	5.638
2.0g Eclipse eSE	125.0	403.1	158.6	244.5	0.393	2.659	0.902	0.747	3.561	4.214	1.427	5.641
4.0g Eclipse eSE	125.0	387.6	149.2	238.4	0.385	2.697	0.945	0.741	3.642	4.018	1.407	5.425
0.75g Eclipse mlSE	124.8	353.8	146.4	207.5	0.415	2.802	0.969	0.744	3.771	4.104	1.420	5.523
1.5g Eclipse mlSE	125.3	378.8	150.6	228.2	0.398	2.593	0.931	0.736	3.524	3.907	1.402	5.310
3.0g Eclipse mlSE	123.3	356.5	148.0	208.5	0.416	2.703	1.006	0.730	3.710	4.002	1.479	5.481
2.0L Moddus mlSE	125.1	343.4	155.9	187.5	0.454	2.891	0.503	0.852	3.394	4.506	0.783	5.290
1.5L M emSE +1.5kg Apogee mlSE	124.7	369.9	167.1	202.8	0.454	2.943	0.547	0.845	3.490	4.912	0.907	5.820
1.5L M + 1.5kg Apogee mlSE	124.0	385.9	153.8	232.1	0.398	2.792	0.535	0.842	3.327	4.286	0.812	5.098
1.5L M + 1.5g Eclipse mlSE	125.1	360.8	158.8	201.9	0.440	3.081	0.541	0.851	3.622	4.891	0.859	5.749
1.5L M + 3.0L Slow Grow mlSE	124.7	275.3	127.4	147.9	0.466	2.901	0.477	0.860	3.378	3.698	0.608	4.306
3.5L Slow Grow eSE	120.3	295.6	172.6	123.0	0.583	2.607	0.795	0.766	3.402	4.506	1.378	5.884
1.5L Slow Grow mlSE	124.7	376.3	145.6	230.7	0.387	2.670	0.943	0.739	3.613	3.886	1.374	5.260
1.5L Sunny eSE	104.8	393.6	140.7	252.9	0.357	2.774	1.149	0.707	3.923	3.895	1.611	5.506
<i>F prob</i>	<i><.001</i>	<i>0.131</i>	<i>0.091</i>	<i>0.002</i>	<i><.001</i>	<i>0.081</i>	<i><.001</i>	<i><.001</i>	<i>0.613</i>	<i><.001</i>	<i><.001</i>	<i>0.128</i>
<i>l.s.d. .05</i>	<i>5.5</i>	<i>n.s.</i>	<i>n.s.</i>	<i>50.5</i>	<i>0.030</i>	<i>n.s.</i>	<i>0.181</i>	<i>0.026</i>	<i>n.s.</i>	<i>0.768</i>	<i>0.362</i>	<i>n.s.</i>

3.4 Discussion

Yield parameters and alkaloid content varied markedly between trial sites. Control treatments in the Hagley trial in 2004-05 produced very high capsule yields, over 30% higher than the average poppy yield in Tasmania, currently around 2.5 t/ha (PACB, 2010). In contrast yield was lower at Symmons and differences can be partly attributed to lower soil water holding capacity as the sodosol soils at this site generally limit crop root depth to 25 cm (Chilvers, 1996). Alkaloid content decreases with rainfall events prior to maturity (Hofman and Menary, 1984) and low alkaloid content at Symmons was likely to be the result of alkaloid leaching from capsules with heavy rains prior to harvest. Oripavine content was particularly low in the Symmons trial and may have been more affected by leaching. In a study conducted by Fist (unpublished), oripavine was recorded in relatively high concentrations in leachate collected after watering.

3.4.1 The effect of PGR application on plant growth

Numerous studies have shown decreased plant height with application of PGRs (e.g. see reviews by Pinthus, 1973; Berry *et al.*, 2004; Rajala, 2004) and growth suppressants (Pinthus, 1973; Hebblethwaite and Burbidge, 1976; Rademacher and Brahm, 2010). In this study, plant height was reduced with applications of Sunny and Ethrel and higher rates of Apogee and Slow Grow. Of these responses, those from Sunny treatment were the most consistent. Data from the preliminary examination of different rates of Sunny at Symmons, 2003–04 suggests an incremental decrease in height with increasing rate; however beyond 1.0 L/ha there may be little additional agronomic or economic benefit. This is consistent with the results of trials conducted by industry and Sunny is now applied commercially at rates up to 1 L/ha (Sumitomo, 2010).

Lack of consistent effects on plant height will limit commercial application of some of the other PGRs. The variation in plant height observed in Ethrel treated plots would cause difficulties with commercial harvesting operations where, to maintain high alkaloid content, it is necessary to minimise the quantity of stem material (Laughlin, 1987). Effects of Ethrel on plant growth have also varied with growth stage at time of application with less effect when applied at bud formation compared with stem elongation (Hsu and Forman, 1982). The effect of Eclipse on plant height was inconsistent across trials with decreased plant height only occurring at Cressy in 2002–

03. In a previous study, Eclipse applied at the rosette growth stage stunted poppy growth but, by mid stem elongation, there was no difference compared with control treatments (Dean, unpublished). Effects of Eclipse applied commercially as a herbicide to poppies can also be variable with efficacy highly dependent on plant growth stage at the time of application. Low rates of Slow Grow had no significant effect on plant height, consistent with a previous study (Forbes and Laughlin, 1985) whereas rates above 5 L/ha stunted growth severely. Yet, application of Slow Grow is recommended at 10L/ha for potatoes and onions (Kendon, 2010). Differential sensitivity appears to be related to the varying ability of plant species to detoxify maleic hydrazide (Swietlinska and Zuk, 1978). Results with application of Cultar on growth of ornamental poppies have also been inconsistent with negligible effects in one trial and a phytotoxic response in another (Martinetti *et al.*, 1994). Variable effects from application of PGRs and other chemicals on plant height are not unusual with responses depending on variables such as crop management, stage of development and weather and growing conditions (Hoffman, 1992; Rademacher, 1990).

Plant height was generally not influenced by application of Moddus, consistent with the initial screening of PGRs in morphine poppies conducted by Dean and Fist in 2001-02 (unpublished) and field observations (A. Fist pers. comm.). However, at Cressy, the highest rate of Moddus applied reduced plant height significantly. This site was planted late (October 3) and it is possible that at the other sites early growth was inhibited but there was sufficient time for compensatory growth in the upper internodes. This “bounce-back” effect has been documented previously and occurs where plants grow out of inhibitory effects as the PGR is metabolised and there are subsequent increased levels of GA biosynthesis (Rademacher, 2000; Rajala, 2004). Alternatively, high temperatures after application of Moddus may have increased efficacy. Maximum daily temperature after PGR application near the Cressy site was as high as 29°C (BOM, 2010). As anti-GA products such as Moddus are reported to be less effective below 8°C (Poole, 2005), it is possible there is greater efficacy at high temperatures that causes increased plant regulatory effects. While Moddus is rapidly absorbed, activation requires conversion to an acidic form (Rademacher, 2000; Spinelli *et al.*, 2010) and this may occur more rapidly with higher temperatures.

Lodging did not occur at any site apart from a low incidence at Hagley and this was limited to control plots. This is consistent with the initial PGR screening trial conducted

by Dean and Fist in 2001-02 (unpublished) where moderate lodging was reduced with application of Ethrel, particularly at higher rates and to a lesser extent with Apogee and Moddus. Application of Moddus also reduced lodging in three paddock trials conducted by Tasmanian Alkaloids in 2004–05 where there was extensive lodging in untreated sections (A. Fist, pers. comm.). Decreased lodging after application of Moddus has also been recorded in wheat (Matysiak, 2006; Zagonel and Fernandez, 2007; Espindula *et al.*, 2009), and ryegrass (Borm and van den Berg, 2008) and can be partly attributed to decreases in plant height and lower centre of gravity (Wiersma *et al.*, 1986; Crook and Ennos, 1995). However, in trials conducted by Batch (1981), application of Cycocel and paclobutrazol to cereals reduced final crop height but decreased lodging had greater correlation with increased stem strength. In other studies application of PGRs has increased stem diameter and reduced lodging in cereals (Tolbert, 1960; Crook and Ennos, 1995; Tripathi *et al.*, 2003) and a similar response may also be occurring after application of Moddus to poppies. Stem diameter in the current study did not differ between treatments (S. Morris, pers. comm.) but is inherently variable in poppies. Nagy *et al.* (1989) reported increased basal stem diameter in poppies after the application of Cultar but the application rate was four-fold higher than this study, an uneconomic rate. To demonstrate differences with lower PGR rates, a greater number of replications with equidistant plant spacing are therefore required. It has also been further suggested by agrochemical companies that reduced lodging after Moddus application in cereals also occurs through increased root growth. Studies with turf grasses (Baldwin *et al.*, 2006; McCullough *et al.*, 2006a) have shown application of Moddus can enhance root mass; however the evidence has been contradictory (McCullough *et al.*, 2005) and there have been no reports of comparable increases in cereals in the scientific literature.

3.4.2 The effect of PGR application on yield components

While seed from morphine poppies is a valuable commodity for the baking and confectionary industries (Duke, 1973; Mahdavi-Damghani *et al.*, 2010), toxicological concern with external residue on seed from the thebaine crop limits the commercial value. Thus a decreased seed yield is beneficial for the industry through reduced processing and transport costs. Slow Grow at rates of 3L/ha and higher, applied both solely and in combination with Moddus, was particularly effective in reducing seed yield with an average reduction of 30% compared with control plots. The large effect of Slow Grow on seed yield has been reported in subsequent trials in poppies (A. Fist pers.

comm.) and also in perennial ryegrass (Hebblethwaite and Burbidge, 1976). Slow Grow is used as a herbicide and applied to turf grass to suppress production of seed heads (Fletcher *et al.*, 2000) and as suggested by Rademacher and Brahm (2010), reduced plant productivity should be expected when applying such compounds. The active ingredient, maleic hydrazide, inhibits the synthesis of nucleic acids and in the plant degrades to hydrazine, a known mutagen (Swietlinska and Zuk, 1978) and both effects are likely to be detrimental to seed development and hence yield.

Application of Ethrel, particularly at higher rates also decreased seed yield and capsule yield and size, consistent with earlier studies (Hsu and Forman, 1982; Dean and Fist, unpublished). Growth stage at time of Ethrel application also influences yield with greater effects at stem elongation in poppies (Hsu and Forman, 1982) and flag leaf stage in barley, the latter due to increased pollen sterility and reduced grain number (Foster and Taylor, 1993). Application of Ethrel can be potentially damaging when the crop is stressed (Poole, 2005) through drought (Knapp *et al.*, 1987) or elevated temperatures resulting in enhanced release of ethylene (Moes and Stobbe, 1991). To ensure product efficacy, Poole (2005) suggests Ethrel should be applied when the temperature is between 15-20°C and, as maximum daily air temperatures after PGR application at the Cressy site exceeded 25°C for a week, this may have impacted on seed development.

Application of Moddus, particularly above 0.75 L/ha also decreased seed yield, consistent with results in an initial PGR trial (Dean and Fist, unpublished). When applied at higher than recommended rates, application of Moddus also decreased grain yield in wheat (Zagonel and Fernandez, 2007; Espindula *et al.*, 2009). Unlike high rates of Slow Grow and Ethrel that tended to reduce straw yield, effects of Moddus were more variable but applied both solely and in all combinations tended to increase straw yield at Hagley. This coupled with effects on seed yield resulted in significant improvements in the S/C ratio. The effects of rate and multiple applications of Moddus on seed and straw yield will be further investigated in Chapter 4.

High rates of Sunny and Apogee applied at mid-late stem elongation, both solely and in combination with Moddus, tended to increase seed yield and decrease the S/C ratio. The increase in seed yield may be a function of reduced plant height with reallocation of assimilates from the stem to seed, as has been attributed to grain yield increases in cereals (Knapp *et al.*, 1987; Rajala and Peltonen-Sainio, 2002). Effects of Sunny on

seed yield were site specific and the lack of response at Symmons could be related to canopy development. It was observed that the higher rates of Sunny applied at Symmons reduced canopy size in addition to plant height so that ground cover was less developed compared with other treatments. In contrast, at Hagley plants grew well and even with application of Sunny there was good canopy closure that tended to result in relatively high seed and straw yields. Effects of Sunny on plant growth and yield components will be further examined in Chapter 5.

3.4.3 The effect of PGR application on alkaloid

The effects of PGRs on alkaloid content and yield were generally variable. There were however consistent responses following application of Moddus and, in particular, very large decreases in oripavine content and yield. Coupled with this, thebaine content and yield tended to increase and the net effect was a highly significant increase in the T ratio and no change in total alkaloid content or yield. Large effects of Moddus on alkaloid composition were also obtained in 19 commercial trials conducted in 2004-05 by Tasmanian Alkaloids in which half of each paddock was sprayed with Moddus and the average increase in thebaine yield was over 30% (A. Fist, pers. comm.). The activity of Moddus on alkaloid content and profile also appears to be consistent as effects were not altered by other PGRs applied in combination with Moddus. The effect of Moddus in altering alkaloid composition will be further investigated in Chapter 4 with different rates and timing of application. Apogee, as with Moddus, is an acylcyclohexanedione PGR and both altered alkaloid profile in an initial screening of PGRs in morphine poppies (Dean and Fist, unpublished). In the current study, effects of Apogee treatment on oripavine and T ratio although tending to be similar, were consistently less than those of Moddus. In particular, in all trials the increase in T ratio after application of Moddus was significantly higher than for Apogee. Rademacher (2000) has suggested that Moddus tends to be less effective in dicotyledonous plants as activation requires conversion to an acidic form that occurs readily in monocotyledons but not in many dicotyledons.

In contrast to the acylcyclohexanedione PGRs, higher rates of Sunny tended to increase oripavine content while maintaining equivalent thebaine levels. Delay in onset of leaf senescence was observed with application of Sunny and this may account for changes in alkaloid content through extending the duration of alkaloid biosynthesis. Similarly, as

oripavine is formed from thebaine, delayed maturity may in particular prolong oripavine biosynthesis. Alternatively, as Sunny was the only PGR to consistently reduce plant height, increased alkaloid content may be a response to stem shortening, with reallocation of plant assimilates from the stem to the alkaloid. Effects of Sunny treatment on alkaloid content and profile are further examined in Chapter 5.

High rates of Ethrel tended to increase total alkaloid with little effect on alkaloid profile. Ethrel treatment has produced similar effects in other studies with poppies (Ramanathan 1978; Buzuk *et al.*, 2005) and has also increased alkaloid and secondary metabolite content in *Catharanthus roseus* (Pan *et al.*, 2010) and *Chrysanthemum cinerariaefolium* Vis. (Haque *et al.*, 2007). Increased total alkaloid with application of Ethrel may also be due to the observed delay in maturity. However, Ethrel also increased alkaloid content in young poppy seedlings and is suggested to enhance translocation (Buzuk *et al.*, 2005) or increase flux of secondary metabolites (Haque *et al.*, 2007). As with effects on growth, alkaloid responses were particularly variable with application of Ethrel, Eclipse and Slow Grow. Effects of Ethrel on alkaloid content have also been variable in other studies (Dean and Fist, unpublished) and responses are highly dependent on season, poppy variety, growth stage and rate of application (Ramanathan, 1978; Hsu and Forman, 1982; Buzuk *et al.*, 2005). Response to application of Eclipse also varied greatly with rate and growth stage; while thebaine content tended to increase with Eclipse application there was a four-fold difference in optimal rate across trials and different growth stages.

In summary, while effects of PGRs in this study were often variable, several responses were consistent and significant, specifically treatments with Moddus (alkaloid profile), Sunny (plant height) and Slow Grow (seed yield). Increased thebaine content is of particular economic interest to the poppy industry and application of Moddus tended to increase thebaine yield through modification of alkaloid composition and increased straw yield. Optimal rate and timing of Moddus and the effect of multiple or split applications were investigated in Chapter 4. While the major effect of Sunny was reduced plant height and potentially less lodging, application also tended to increase oripavine content while maintaining thebaine levels. Effects on yield components, seed in particular, appeared to be related to site growing conditions with relatively low yields where application of PGR had reduced canopy size. To further evaluate this and to

induce conditions suitable for lodging, Sunny treatments in a subsequent study (Chapter 5) were applied to different canopy sizes generated through a range of plant populations.

CHAPTER 4. THE EFFECT OF APPLICATION RATE AND TIMING OF THE PLANT GROWTH REGULATOR MODDUS ON POPPIES

4.1 Introduction

Previous studies in poppies have shown beneficial responses in alkaloid content following application of PGRs. In particular, use of triacontanol (Srivastava and Sharma, 1990; Khan *et al.*, 2007) and Ethrel (Ramanathan, 1978; Ramanathan, 1981; Buzuk *et al.*, 2005) have increased morphine content. In the preliminary trial of the potential use of PGRs in morphine poppies in 2001-02 (Dean and Fist, unpublished), two acylcyclohexanedione PGRs, Moddus and Apogee, tended to alter alkaloid profile resulting in a greater proportion of thebaine and less morphine. Further screening trials (Chapter 3) showed that oripavine content decreased while thebaine content increased with the application of Moddus. As there were no significant effects on total alkaloid content, Moddus appears to act through a different mechanism and it was proposed by Dean *et al.* (2005) that the biosynthesis of oripavine from thebaine is inhibited (see Figure 2.2 for morphinan alkaloid pathway). Recently it has been confirmed that 2-oxoglutarate-dependent dioxygenase enzymes are required for the demethylation of thebaine to morphine in poppies (Hagel and Facchini, 2010). The authors further suggest that Moddus-induced inhibition of dioxygenases increases thebaine content.

Moddus is also applied to cereals and ryegrass to reduce plant height and lodging (Matysiak, 2006; Zagonel and Fernandez, 2007; Borm and van den Berg, 2008; Espindula *et al.*, 2009), retard growth in turf grasses (McCullough *et al.*, 2007) and increase sugar content in sugarcane (Rixon *et al.*, 2007). An increase in seed yield has also been reported with the use of Moddus (Matysiak, 2006; Zagonel and Fernandez, 2007; Borm and van den Berg, 2008; Espindula *et al.*, 2009). However, efficacy of PGR application on plant growth and yield is affected by the rate applied. For example a linear reduction in plant height has been recorded in cereals with increasing rate of Moddus (Matysiak, 2006; Zagonel and Fernandez, 2007; Espindula *et al.*, 2009), Cycocel (Perry and Miers, 1986) and Ethrel (Nafziger *et al.*, 1986; Foster *et al.*, 1991). Moddus at rates marginally less than twice the recommended rate decreased grain yield

in wheat (Zagonel and Fernandez, 2007; Espindula *et al.*, 2009). Similarly, PGR effects on grain yield components and height of cereals are also influenced by plant growth stage at the time of treatment. Applications at early tillering tended to increase ears/m² (Waddington and Cartwright, 1986; Ma and Smith, 1991; Hussain and Leitch, 2007) whereas the number of grains/ear was enhanced with applications at the start of stem elongation (Waddington and Cartwright, 1986; Naylor, 1989; Hussain and Leitch, 2007). Later applications of Moddus at early-mid stem elongation in wheat (GS32-33) were more effective in reducing stem height than an early stem elongation treatment (GS31-32; Zagonel and Fernandez, 2007; Espindula *et al.*, 2009).

Given the economic importance of increased thebaine content, more detailed trials were conducted to determine whether rate and timing of Moddus application influence the growth, yield and, in particular, alkaloid content of thebaine poppies. To determine if effects on alkaloid are cumulative, studies on optimal rate were further extended by assessing responses to multiple and split applications of Moddus.

4.2 Materials and Methods

4.2.1 Trial design and treatments

Three field trials were conducted in northern Tasmania in 2003-04 (Symmons) and 2004-05 (Hagley; Symmons) to examine the effect of different rates and timing of Moddus application on growth, yield and alkaloid content of thebaine poppies. Details of rates and growth stages at application timing for single, split and multiple treatments are listed in Table 4.1. Based on preliminary trial data (Chapter 3), application rates of Moddus trialled in the 2003-04 trial ranged from 1.0 L/ha to 4.0 L/ha and targeted growth stages from early stem elongation to mid flowering. In trials in 2004-05 the range of rates was narrowed to 1.5 to 3.0 L/ha and the earlier growth stage excluded. (See Figure 2.1 for illustration of growth stages of poppy plant). The trial at Symmons in 2004-05 was largely conducted for farmer demonstration purposes and with refinement of appropriate rates and timing of application, there were fewer treatments. At Symmons in 2003-04, double applications were applied with 2 L/ha applied at early stem elongation followed by applications of 1.0, 2.0 or 4.0 L/ha at flowering. At Hagley and Symmons in 2004-05, multiple rates of Moddus were equal in total to single rates to

allow comparison of the same rate split across different timings. At Symmons, double applications totalled 1.5 L/ha (2 x 0.75 L) and 2.5 L/ha (2 x 1.25 L) and at Hagley double and triple treatments totalled 2.0 L/ha (2x 1.0 L, 3 x 0.67 L) and 3.0 L/ha (2 x 1.5 L, 3 x 1.0 L). Experimental designs were rate x timing factorials plus control with additional multiple or split rate treatments and 3 - 4 replicates.

Table 4.1. Rates and timing of single and multiple applications of Moddus in field trials, 2003-2005.

Site / Year	Applications	Rates and total applied (L/ha)	Times of application			
			eSE	emSE	mlSE	flwr
Symmons (2003-04)	single (3 rates x 3 times)	1.0, 2.0 or 4.0 L	X		X	X
	double	2.0 L + 1.0, 2.0 or 4.0 L	X			X
Hagley (2004-05)	single (4 rates x 3 times)	1.5, 2.0, 2.5 or 3.0 L		X	X	X
	double (as split)	2.0 L (2 x 1.0); 3.0 L (2 x 1.5)		X	X	
	triple (as split)	2.0 L (3 x 0.67); 3.0 L (3 x 1.0)		X	X	X
Symmons (2004-05)	single (3 rates x 2 times)	1.5, 2.0 or 2.5 L		X		X
	double (as split)	1.5 L (2 x 0.75); 2.5 L (2 x 1.25)		X		X

For timing of application SE = stem elongation; e = early; em, early to mid; ml, mid to late; flwr, flowering

4.2.2 Cultural details, measurements and processing

Field site and trial information are presented in Table 4.2. Plant height was not measured as visually there were no differences in the current trials and Moddus treatment did not generally affect plant height in the PGR screening (Chapter 3). Cultural details and information on measurements, harvest procedure, processing and analysis of samples has been previously outlined in Chapter 3. Being thebaine poppies, only thebaine and oripavine alkaloids were analysed.

4.2.3 Statistical analysis

The significance of rates of Moddus, times of application and their interactions were analysed using a two-way ANOVA in Genstat version 12.1. To enable comparison with the nil treatment a control structure was added to the factorial. An additional two-way ANOVA analysis was conducted to compare the effect of multiple and split applications

with single treatments. When F probabilities were significant ($P < 0.05$) the least significant difference (l.s.d.) was calculated at $P = 0.05$ for testing differences between means.

Table 4.2. Site and trial information for Moddus PGR trials in thebaine poppies, 2003-2005.

Year	2003-04	2004-05	
Site	Symmons	Hagley	Symmons
Soil type	sodosol	dermosol	sodosol
Previous crop	lupins	onions	pasture
Replicates	3	4	3
Sowing date	9 Sep 2003	18 Aug 2004	22 Jul 2004
rate (g/ha)	800	800	700
Basal N:P:K fertiliser	10:17:8 + B	10:17:8	14:16:11 + B
rate (kg/ha)	400	400	370
Harvest date	9 Feb 2004	25 Jan 2005	11 Jan 2005
area/plot	16.2 m ²	14.8 m ²	7.2 m ²

4.3 Results

Monthly rainfall and temperature data is presented in Appendix I and further details on site and growing conditions for Symmons, 2003-04 and Hagley, 2004-05 in Chapter 3. Soil crusting reduced plant density at Symmons in 2004-05 whereas establishment was optimal at other sites. There were very high rainfall events at Symmons in January 2004 with 109 mm falling over the 2 week period prior to harvest. There were no interactions between rate and timing in any measured parameters and thus only main effects are described. Data on timing of application from the rate x timing analyses were generally similar to that from the comparison of single and multiple or split rates and to avoid duplication only this latter data set is presented.

4.3.1 Trial at Symmons, 2003-04

4.3.1.1 The effect of Moddus application on growth and yield components

Comparison of pooled single Moddus treatments with control plots showed no significant effects ($P > 0.05$) on mean capsule weight and yield of capsules, straw and seed (Table 4.3). However Moddus application tended to increase straw and reduce seed yield compared with the control and consequently the S/C ratio increased significantly (Table 4.3). Similarly, there were no significant main effects of rate and timing of Moddus on yield components apart from an increase in the S/C ratio with later application timing and multiple treatments. Higher rates also tended ($P = 0.08$) to increase the S/C ratio.

4.3.1.2 The effect of Moddus application on alkaloid

Single applications of Moddus increased thebaine content and yield and decreased oripavine content and yield (Table 4.3). Consequently total alkaloid content and yield did not differ from the control. Rate of Moddus application had no effect ($P > 0.05$) on thebaine and total alkaloid content whereas oripavine content was decreased and the T ratio increased. Application of Moddus at early stem elongation showed the least effect on alkaloid content with the lowest thebaine content and a trend towards higher oripavine content (Table 4.3) compared with later applications. These responses were also expressed in a significantly lower T ratio. Multiple applications of Moddus increased the thebaine content and yield and reduced oripavine content compared with single applications but differences were generally only significant relative to the early application.

4.3.2 Trial at Hagley, 2004-05

4.3.2.1 The effect of Moddus application on growth and yield components

Pooled single application of Moddus increased straw yield by 8% and decreased seed and capsule yields compared with the control (Table 4.4). Consequently there was a large increase in the S/C ratio. There were no significant main effects of rate and timing on straw and capsule yield. However delay in timing of application tended to decrease straw yield and coupled with increased seed yield, this resulted in a lower S/C ratio (Table 4.4). Higher rates also tended to increase the S/C ratio ($P = 0.07$). Both double and triple split rate applications did not affect straw yield ($P > 0.05$) but decreased seed

yield and increased the S/C ratio compared with a single application at flowering. Seed yield of the triple treatment in particular was decreased below that of all single applications and the S/C ratio was higher than all other treatments.

4.3.2.2 The effect of Moddus application on alkaloid

Single applications of Moddus increased thebaine content and yield by, on average 15% and 24%, respectively, compared with the control (Table 4.4). Conversely oripavine content and yield decreased by 47% and 42%, respectively, compared with the control, which resulted in a large increase in the T ratio. Triple applications of Moddus increased thebaine content and yield, while rate and timing of application showed no significant effect (Table 4.4). The triple treatment also tended to increase total alkaloid content compared with single treatments. Oripavine content tended to decrease with higher rates and was significantly lower at the mid-late stem elongation growth stage resulting in a greater T ratio.

4.3.3 Trial at Symmons, 2004-05

Single Moddus treatments reduced seed yield and thus increased the S/C ratio and the double application of Moddus further enhanced these effects although the S/C ratio was not significant ($P = 0.14$). Single applications of Moddus increased thebaine content and yield by 18% and 25%, respectively (Table 4.5) whereas oripavine content and yield decreased resulting in no significant difference in total alkaloid content and yield. Thebaine content increased with split application and this was also reflected in a trend towards higher total alkaloid content ($P = 0.11$) whereas rate and timing of a single application of Moddus had no significant effect. The lowest rate of Moddus (1.5 L/ha) resulted in higher oripavine content and a lower T ratio (Table 4.5).

Table 4.3. Effect of different rates and timing of single and multiple applications of Moddus on yield and alkaloid parameters at Symmons, 2003-04. Data analyses consist of main effects of (1) PGR data from comparison of pooled single applications of Moddus with control plots (2) Rate data from rate x timing analyses (3) Timing data from comparison of single and multiple rates.

Treatment	Capsule wt (g)	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Total alk yld (g/m ²)
1. PGR												
Control	4.44	215.3	72.1	143.2	0.336	1.842	0.574	0.763	2.416	1.332	0.417	1.750
Moddus	4.15	206.9	76.7	130.3	0.371	2.168	0.348	0.863	2.516	1.671	0.268	1.939
<i>F prob</i>	<i>0.157</i>	<i>0.567</i>	<i>0.387</i>	<i>0.188</i>	<i><.001</i>	<i><.001</i>	<i><.001</i>	<i><.001</i>	<i>0.337</i>	<i>0.017</i>	<i>0.001</i>	<i>0.266</i>
<i>l.s.d.</i> _{0.05}	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.012</i>	<i>0.156</i>	<i>0.070</i>	<i>0.017</i>	<i>n.s.</i>	<i>0.272</i>	<i>0.081</i>	<i>n.s.</i>
2. Rate												
1.0L/ha	4.250	210.5	75.7	134.4	0.363	2.135	0.409	0.840	2.544	1.617	0.309	1.926
2.0L/ha	4.167	199.4	75.2	124.0	0.377	2.209	0.358	0.862	2.567	1.681	0.274	1.955
4.0L/ha	4.019	211.1	79.0	132.4	0.374	2.159	0.277	0.887	2.436	1.716	0.222	1.938
<i>F prob</i>	<i>0.560</i>	<i>0.697</i>	<i>0.776</i>	<i>0.562</i>	<i>0.080</i>	<i>0.666</i>	<i>0.003</i>	<i><.001</i>	<i>0.468</i>	<i>0.792</i>	<i>0.096</i>	<i>0.987</i>
<i>l.s.d.</i> _{0.05}	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.070</i>	<i>0.017</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
3. Timing												
eSE	4.204	196.0	71.5	124.5	0.365	2.046	0.385	0.843	2.431	1.475	0.280	1.755
mlSE	4.009	212.1	78.1	133.9	0.369	2.234	0.298	0.883	2.532	1.751	0.233	1.984
flwr	4.222	212.8	80.3	132.4	0.379	2.224	0.361	0.862	2.585	1.787	0.292	2.079
eSE + flwr	4.156	212.8	83.0	129.8	0.392	2.333	0.281	0.893	2.614	1.931	0.235	2.167
<i>F prob</i> _{0.05}	<i>0.556</i>	<i>0.591</i>	<i>0.192</i>	<i>0.764</i>	<i><.001</i>	<i>0.020</i>	<i>0.009</i>	<i><.001</i>	<i>0.379</i>	<i>0.031</i>	<i>0.247</i>	<i>0.137</i>
<i>l.s.d.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.012</i>	<i>0.173</i>	<i>0.065</i>	<i>0.016</i>	<i>n.s.</i>	<i>0.295</i>	<i>n.s.</i>	<i>n.s.</i>

Growth stages at times of application: SE, stem elongation; e, early; ml, mid to late; flwr, flowering.

Table 4.4. Effect of different rates and timing of single and split applications of Moddus on yield and alkaloid parameters at Hagley, 2004-05.

Data analyses consist of main effects of (1) PGR data from comparison of pooled single applications of Moddus with control plots (2) Rate data from rate x timing analyses (3) Timing data from comparison of single and split rates.

Treatment	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Total alk yld (g/m ²)
1. PGR											
Control	274.4	104.3	170.1	0.380	2.600	0.949	0.732	3.549	2.719	0.991	3.710
Moddus	255.3	112.9	142.5	0.443	2.984	0.506	0.855	3.490	3.371	0.572	3.943
<i>F prob</i>	<i>0.003</i>	<i>0.002</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>0.287</i>	<i>< .001</i>	<i>< .001</i>	<i>0.069</i>
<i>l.s.d. 0.05</i>	<i>12.2</i>	<i>5.15</i>	<i>8.16</i>	<i>0.103</i>	<i>0.097</i>	<i>0.044</i>	<i>0.012</i>	<i>n.s.</i>	<i>0.211</i>	<i>0.062</i>	<i>n.s.</i>
2. Rate											
1.5L/ha	262.6	115.3	147.4	0.439	2.958	0.531	0.848	3.489	3.409	0.615	4.025
2.0L/ha	258.5	112.4	146.0	0.435	2.924	0.534	0.846	3.458	3.285	0.600	3.886
2.5L/ha	252.7	114.2	138.5	0.453	3.040	0.478	0.864	3.518	3.480	0.550	4.030
3.0L/ha	249.1	110.9	138.2	0.446	3.022	0.480	0.863	3.503	3.357	0.531	3.888
<i>F prob</i>	<i>0.383</i>	<i>0.613</i>	<i>0.223</i>	<i>0.074</i>	<i>0.253</i>	<i>0.115</i>	<i>0.038</i>	<i>0.865</i>	<i>0.588</i>	<i>0.149</i>	<i>0.725</i>
<i>l.s.d. 0.05</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.016</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
3. Timing											
emSE	252.1	115.8	136.2	0.460	3.019	0.479	0.863	3.498	3.494	0.555	4.048
mlSE	249.9	110.8	139.1	0.443	2.933	0.437	0.871	3.369	3.249	0.485	3.734
flwr	259.4	108.3	151.0	0.418	2.968	0.606	0.831	3.574	3.220	0.657	3.878
emSE + mlSE	240.4	111.5	128.9	0.464	3.076	0.451	0.872	3.527	3.430	0.503	3.933
emSE +mlSE +flwr	233.8	113.3	120.2	0.486	3.215	0.486	0.868	3.701	3.641	0.553	4.193
<i>F prob</i>	<i>0.046</i>	<i>0.384</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>< .001</i>	<i>0.002</i>	<i>0.039</i>	<i>< .001</i>	<i>0.123</i>
<i>l.s.d. 0.05</i>	<i>17.4</i>	<i>n.s.</i>	<i>10.3</i>	<i>0.009</i>	<i>0.120</i>	<i>0.039</i>	<i>0.008</i>	<i>0.146</i>	<i>0.295</i>	<i>0.070</i>	<i>n.s.</i>

Growth stages at times of application: SE, stem elongation; e, early; ml, mid to late; flwr, flowering.

Table 4.5. Effect of different rates and timing of single and split applications of Moddus on yield and alkaloid parameters at Symmons, 2004-05. Data analyses consist of main effects of (1) PGR data from comparison of pooled single applications of Moddus with control plots (2) Rate data from rate x timing analyses (3) Timing data from comparison of single and split rates.

Treatment	Capsule yld (g/m ²)	Straw yld (g/m ²)	Seed yld (g/m ²)	Straw/Caps ratio	Thebaine %	Oripavine %	Thebaine ratio	Total alk %	Theb yld (g/m ²)	Ori yld (g/m ²)	Total alk yld (g/m ²)
1. PGR											
Control	131.8	53.6	78.3	0.406	2.236	1.256	0.640	3.492	1.199	0.673	1.872
+PGR	128.5	56.7	68.0	0.455	2.648	0.752	0.779	3.401	1.503	0.426	1.929
<i>F prob</i>	<i>0.115</i>	<i>0.206</i>	<i>< .001</i>	<i>< .001</i>	<i>0.001</i>	<i>< .001</i>	<i>< .001</i>	<i>0.458</i>	<i>0.008</i>	<i>< .001</i>	<i>0.659</i>
<i>l.s.d.</i> _{0.05}	<i>n.s.</i>	<i>n.s.</i>	<i>6.05</i>	<i>0.169</i>	<i>0.212</i>	<i>0.089</i>	<i>0.019</i>	<i>n.s.</i>	<i>0.249</i>	<i>0.096</i>	<i>n.s.</i>
2. Rate											
1.5L/ha	119.9	54.3	65.6	0.453	2.624	0.826	0.761	3.450	1.426	0.448	1.875
2.0L/ha	128.5	59.0	69.5	0.459	2.715	0.756	0.782	3.470	1.608	0.446	2.053
2.5L/ha	125.5	56.7	68.8	0.452	2.605	0.676	0.794	3.280	1.476	0.383	1.859
<i>F prob</i>	<i>0.128</i>	<i>0.133</i>	<i>0.200</i>	<i>0.561</i>	<i>0.490</i>	<i>0.012</i>	<i>0.011</i>	<i>0.247</i>	<i>0.149</i>	<i>0.136</i>	<i>0.220</i>
<i>l.s.d.</i> _{0.05}	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>0.083</i>	<i>0.018</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
3. Timing											
emSE	123.0	56.2	66.9	0.456	2.569	0.783	0.767	3.352	1.443	0.438	1.881
flwr	122.3	54.8	67.5	0.448	2.660	0.719	0.788	3.379	1.459	0.393	1.853
emSE + flwr	111.8	52.0	59.8	0.465	2.869	0.749	0.793	3.618	1.489	0.387	1.876
<i>F prob</i>	<i>0.067</i>	<i>0.249</i>	<i>0.028</i>	<i>0.139</i>	<i>0.023</i>	<i>0.418</i>	<i>0.028</i>	<i>0.109</i>	<i>0.828</i>	<i>0.156</i>	<i>0.953</i>
<i>l.s.d.</i> _{0.05}	<i>n.s.</i>	<i>n.s.</i>	<i>5.9</i>	<i>n.s.</i>	<i>0.205</i>	<i>n.s.</i>	<i>0.019</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Growth stages at times of application: SE, stem elongation; em, early to mid; flwr, flowering.

4.4 Discussion

Yield parameters and alkaloid content varied considerably between trials. As discussed in Chapter 3, variation in capsule yields between Hagley and the Symmons sites can be partly attributed to differences in soil water holding capacity. Lower capsule yields in 2004-05 at Symmons were also a result of reduced plant density due to soil crusting after sowing, a common problem with establishment of poppies. The low alkaloid content at Symmons in 2003-04 is likely to be due to the high rainfall events prior to harvest that leach alkaloid from capsule walls (Hofman and Menary, 1984). There were no interactions between either rate and timing or rate and split applications in any measured parameters. Consequently only main effects are described below.

4.4.1 The effect of Moddus application on growth and yield components

Application of Moddus had no visual effect on plant height in comparison with control plots and this is in agreement with the PGR screening trials (Chapter 3) and earlier work (Dean and Fist, unpublished) in which Moddus generally had little effect on reducing plant height. In contrast, height reductions after application of Moddus have been reported in wheat (Matysiak, 2006; Zagonel and Fernandez, 2007; Espindula *et al.*, 2009), rice (Nascimento *et al.*, 2009) and ryegrass (Borm and van den Berg, 2008). Registration of Moddus is restricted to cereals, turf grass, ryegrass and sugar cane suggesting high efficacy is limited to gramineous species. Rademacher (2000) reports that Moddus tends to be less effective in dicotyledonous plants as activation requires conversion to an acidic form that occurs readily in monocotyledons but not in many dicotyledons.

Single Moddus treatments reduced seed yield (mean of 13 %) in all trials compared with control plots although this effect was not significant at Symmons, 2003-04. This is in agreement with the PGR screening trials (Chapter 3) where seed yield was consistently lower in Moddus treated plots. Multiple and split applications further reduced seed yield compared with single applications suggesting that effects are cumulative. Thus, when applied as split applications in 2004-05, the triple treatment at Hagley and the double treatment at Symmons reduced seed yield significantly compared with single

applications. The seed yield of the double treatment at Hagley was also significantly lower than the single application at flowering.

The reduced seed yield in these studies is in contrast with research in other species where application of Moddus has often resulted in grain yield increases in wheat (Matysiak, 2006; Zagonel and Fernandez, 2007; Espindula *et al.*, 2009) and ryegrass (Borm and van den Berg, 2008). Differences in response may relate to the very high rates applied in the current trials compared with the recommended rates of 0.2-0.4 L/ha used in cereals. Grain yield has tended to decrease after application of Moddus at rates only 50% higher than the maximum recommended rate, 0.4 L/ha, in wheat (Zagonel and Fernandez, 2007; Espindula *et al.*, 2009) and rice (Nascimento *et al.*, 2009). The comparatively high base rates of Moddus used in these trials may also have precluded responses to increased rate.

Generally, Moddus applied between early and mid stem elongation appeared to have a greater effect on reducing seed yield than at flowering. However, this effect was only significant at Hagley. PGRs have also affected seed development in other studies when applied at critical times. For example, in barley, applications of Ethrel at or near flag leaf stage may reduce grain numbers due to ethephon-induced pollen sterility (Foster and Taylor, 1993). The comparatively high rates of Moddus applied in the current trials may predispose the plant to similar detrimental effects on seed development at specific growth stages.

Although less evident, application of Moddus also tended to increase straw yield and across all trials there was a consistent and highly significant increase in the S/C ratio. Increased grain yield from PGR application has been attributed in many studies to shorter stems and a redistribution of assimilates from the stem to grain (e.g. Knapp *et al.*, 1987; Rajala and Peltonen-Sainio, 2002). Increase in straw yield with Moddus application may be a result of similar partitioning from stems to capsules. However, as there was little measured (Chapter 3) or visible (this study) decrease in plant height, it is instead possible that reallocation was from the seed, the total yield of which was reduced, to the capsule wall. A similar effect may also occur with the herbicide clopyralid in poppies that is applied commercially at rosette to early stem elongation growth stages to decrease seed and increase straw yield (Scott, 1991). In cereals

concurrent with reduction in plant height, PGRs have also increased stem diameter (Tolbert, 1960; Crook and Ennos, 1995; Tripathi *et al.*, 2003), stem wall thickness (Tripathi *et al.*, 2003) and leaf width and thickness (Gao *et al.*, 1987) and a similar response may occur in poppy capsule walls as well as stems.

4.4.2 The effect of Moddus application on alkaloid

Single applications of Moddus in all three trials increased thebaine and decreased oripavine content and yield compared with control plots. The mean increase in thebaine yield from single Moddus applications was consistently around 25%. Moddus application strongly influenced the proportion of thebaine to oripavine and effects were highly significant in all trials. Moddus and other acylcyclohexanedione PGRs are structurally similar to 2-oxoglutaric acid, a co-substrate for dioxygenase activity. Inactivation of these enzymes inhibits GA biosynthesis (Rademacher, 2000; Kang *et al.*, 2010) but can also affect other plant biochemical pathways. The *top1* poppy mutant used in thebaine poppy varieties in Tasmania accumulates thebaine and oripavine instead of morphine and codeine (Fist, 2001; Millgate *et al.*, 2004). This genotype inhibits demethylation of thebaine to neopinone and in an alternative pathway, oripavine to morphinone (Millgate *et al.*, 2004). Recently Hagel and Facchini (2010) have isolated two oxoglutarate-dependent dioxygenases, thebaine 6-O-demethylase and codeine O-demethylase that catalyse these steps. They also link the increase in thebaine content following acylcyclohexanedione PGR application to inhibition of 2-oxoglutarate-dependent dioxygenase activity.

PGRs may similarly affect dioxygenase activity in other biochemical pathways thereby altering secondary metabolite composition. Inhibition of 2-oxoglutarate-dependent dioxygenases by acylcyclohexanedione PGRs alters the profile of flavonoids resulting in biosynthesis of luteoforal. This phenolic has biocidal properties and can reduce activity of several diseases in apples (Roemmelt *et al.*, 2003; Rademacher *et al.*, 2006; Spinelli *et al.*, 2010). Three major alkaloids are produced in the medicinal plant *Catharanthus roseus* L., vincristine and vinblastine via the monoterpenoid pathway and ajmalicine through another branch (Facchini and St-Pierre, 2005, Mahroug *et al.*, 2007). In a study by Jaleel *et al.* (2006), application of the triazole PGR, triadimefon, resulted in increased ajmalicine content. A monooxygenase is required in the biosynthesis of

vincristine and vinblastine (Mahroug *et al.*, 2007) and as this enzyme is inhibited by triazole PGRs (Rademacher, 2000), increased metabolic flux could be expected to lead to increased levels of ajmalicine. In the same species application of Cycocel increased vinblastine but reduced vindoline content (Pan *et al.*, 2010) and it is feasible that Cycocel may also alter alkaloid composition by inhibiting cyclase enzymes (Rademacher, 2000).

Different rates and times of Moddus application resulted in significant differences in oripavine content. At higher rates of Moddus, oripavine content was decreased and T ratio increased. Moddus applied at mid-late stem elongation resulted in significantly lower oripavine content and a higher T ratio. Analysis of data from trials subsequently conducted by Tasmanian Alkaloids has also shown higher oripavine content after application of Moddus at late stem elongation compared with that at full flower stage (A Fist pers. comm.). The greater efficacy of Moddus in decreasing oripavine at mid-late stem elongation also explains the lack of response to split applications that ranked intermediate between values for single applications. By splitting the rate so that only half (in double treatments) or one-third (in triple treatments) was applied at the most sensitive growth stage, the efficacy of split rates in reducing oripavine is lessened, whereas for all other measured variables, the triple treatment, in particular, produced far larger effects. This explanation also accounts for the lack of response to the additional application at Symmons, 2003-04. In this trial, applications of the multiple treatment were applied at early stem elongation and flowering. Consequently, despite the high rates used at these growth stages, there was no further decrease in oripavine content compared with the single mid-late stem elongation application.

Whereas thebaine was little influenced by rate and timing of Moddus application, the triple split rate treatment at Hagley and double treatment at Symmons increased thebaine content compared with single applications. The double treatment at Hagley also increased thebaine content but was only significantly greater than the single application at mid-late stem elongation. These results suggest that growth stage at time of Moddus application is less critical for thebaine content and importantly, applications at different times are cumulative in effect. This is further supported by the significantly higher thebaine content in the triple treatment compared with the double. Of commercial importance, yield of thebaine also tended to increase with split and multiple

rates, which was significant for the triple treatment at Hagley and multiple rate at Symmons, 2003-04 (10% and 16% higher than single applications, respectively).

It is postulated that Moddus increases thebaine content through inhibiting 2-oxoglutarate-dependent dioxygenases that catalyse demethylation of thebaine to oripavine. However, this does not account for the effects of multiple applications in consistently tending to increase total alkaloid content through increases in thebaine content that occurred in the absence of decreases in oripavine content. Moddus could also be acting to increase flux of morphinan pathway precursors. As with capsule wall growth, increased alkaloid production with split applications may be associated with the large decrease in seed yield and reduced size of competing sinks for plant assimilates. Simplistically, while there was on average a 13% decrease in seed yield the increase in straw yield was only around half of this figure, potentially allowing a proportion of the remainder to be utilised for alkaloid biosynthesis. Reduced demand by seed may therefore allow redirection of metabolite, sucrose and glucose-6-phosphatate to alkaloid production.

CHAPTER 5. THE EFFECT OF SOWING RATE AND SPLIT APPLICATION OF SUNNY ON MORPHINE POPPIES

5.1 Introduction

In Chapter 3, a range of PGRs including those currently used in cereal, grass seed, canola and cotton crops were applied in screening trials to thebaine poppies to assess their effect on plant growth, yield and alkaloid content over three years. Of these PGRs, only the triazole, Sunny, showed consistent effects in reducing plant height. Sunny is applied commercially to reduce vegetative growth in fruit trees and ornamental species (Fletcher *et al.*, 2000; Rademacher and Brahms, 2010) and to reduce height and control lodging in rice (Rademacher 2000; Rademacher and Brahm, 2010). In the screening trials, a number of other triazoles with PGR activity, Cultar, Folicur, Caramba and Opus were also evaluated. However height and yield responses were inconsistent and similar to variable effects found in an earlier study with Cultar applied to poppies (Nagy *et al.*, 1989). Effects of triazoles applied as PGRs can be very specific as evidenced by the narrow efficacy range of Folicur and Caramba on canola (Kightley, 2001; Dapprich *et al.*, 2002) and Opus on cleavers, *Galium aparine* (Benton and Cobb, 1995).

Application of Sunny to thebaine poppies in the screening trials also tended to increase oripavine content while maintaining thebaine levels. Sunny treatment delayed maturity and, as suggested in Chapter 3, this may extend alkaloid biosynthesis and the demethylation of thebaine to oripavine. Morphine is the final alkaloid of the morphinan pathway in morphine poppies (Dewick, 2002; Hagel and Facchini, 2010) and likely to also increase with delayed maturity. Being of greater value to the poppy industry than oripavine, further studies thus focused on application of Sunny to morphine rather than thebaine poppies.

In a trial conducted in 2005-06 (Dean, unpublished), three rates of Sunny (0.5, 1.0 and 1.5 L/ha) were applied at three growth stages (late rosette, mid stem elongation and hook) to morphine poppies. Application at the late rosette stage increased morphine content while later applications tended to increase straw and seed yield. There was no significant effect of rate on yield or alkaloid, however higher rates tended to improve

the proportion of capsule straw. Furthermore, splitting the active ingredient across three times of application increased straw yield more than an equivalent single application. Given the economic benefit of this result and the increased thebaine content with multiple and split applications of Moddus, further work is required to confirm the benefit of split applications of Sunny in morphine crops.

In Chapter 3 the effects of Sunny tended to be influenced by growing conditions with higher yield components than control plots at sites with greater yield potential and lower yields where optimal canopy growth was reduced by application of Sunny. Furthermore, in the 2005-06 trial (Dean, unpublished), the reduced plant height and canopy size with application of Sunny tended to decrease light interception post flowering and was likely to have reduced yield potential.

As the benefit, or otherwise, of PGR application appears to be related to the size of the poppy crop canopy, the aim of this research was to further investigate the interaction between these variables. To generate a range of crop canopy sizes, sowing rate was varied and Sunny treatments applied across sowing rates. Effects on growth, yield and alkaloid content of morphine poppies were measured. In addition, the response to applications split over three growth stages was compared with a single application.

5.2 Materials and methods

5.2.1 Field trial design and treatments

The plant growth, yield and alkaloid content of a morphine poppy variety (WF03-2024) were evaluated across four plant densities and three PGR treatments in a field trial sown on 3 September 2008 at Cressy (41° 45'S; 147° 03'E) in northern Tasmania. Different plant densities were derived from sowing rates of 400, 800, 1200 and 1600 g/ha of seed. Plots were 5 m long by 1.5 m wide and were sown with a cone seeder. Sunny PGR treatments totalled 1.0 L/ha and were either applied as a single application at late rosette stage or split applications of 0.33 L/ha at 3 growth stages: late rosette, mid stem elongation and start of flowering (see Figure 2.1 for illustration of growth stages of the

poppy plant). The trial was a factorial design with four replicates of each plant density and PGR treatment.

5.2.2 Cultural details, measurements and processing

The trial was sown on a black vertosol soil type with 200 kg/ha of 14:16:11 NPK fertiliser + 1.4 kg/ha of boron, following two years of a ryegrass seed crop. Further cultural details are provided in Chapter 3. Plant density was recorded after establishment and at harvest. The latter measurement included the density of small plants with under-developed capsules. Several measures and scores for maturity across the plots were taken: % open flowers at the start of flowering and mid-late flowering and % green capsules at stripey capsule stage prior to senescence. As visual differences between treatments were also evident at the start of flowering, an additional score of plant colour, dark green (1) through to pale green (4), was recorded. Scores of lodging were taken at 3 dates: when lodging first commenced at mid flowering, mid-late flowering and finally at harvest. Incidence of lodging was based on an index adapted from the method of Berry *et al.* (2003):

$$\text{Lodging index} = 1/3a + 2/3b + c$$

where *a*, *b* and *c* are the percentage of plants leaning at angles between 10° - 22°, 22° - 45° and 45° - 90° respectively.

At maturity, the height to the top of the capsule for the main stem and each branch (primary, secondary, tertiary and quaternary) of six plants was measured (Figure 5.1) and the number of capsules recorded at each inflorescence level. Plants had up to four branches but there were insufficient data to analyse beyond the third branch. To determine differences in canopy size, tube solarimeters (Delta T, Cambridge, UK) were used at mid flowering to measure intercepted radiation for the Sunny split application and control treatments at the lowest and highest sowing rate.

Trial plots were harvested by hand on 10 February 2009. To reduce variation, 2 m² of the most uniform section of the plot was harvested. The procedure for harvest, processing and analysis of samples has been outlined previously in Chapter 3. Being morphine poppies, only morphine and codeine alkaloids were analysed.

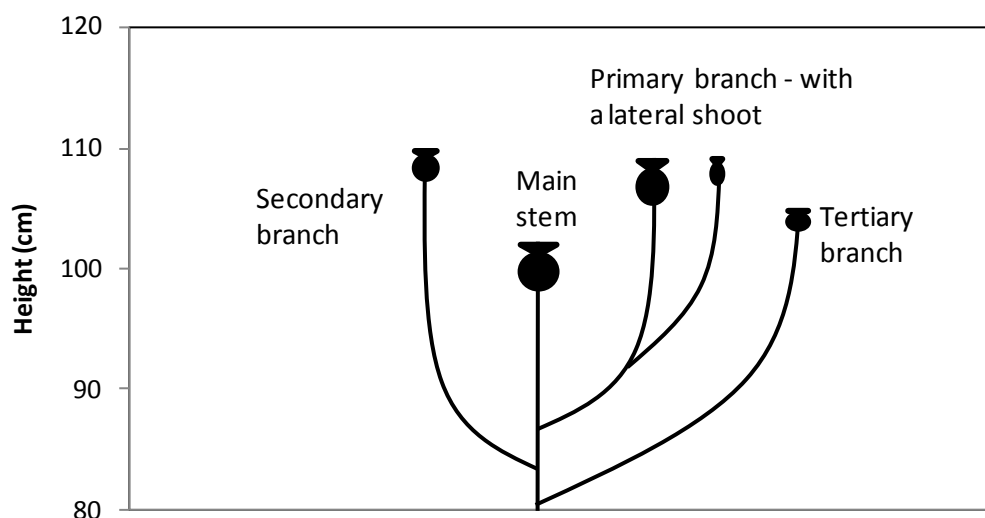


Figure 5.1. Main stem and branching in poppies with mean heights at Cressy, 2008-09.

5.2.3 Statistical analysis

The significance of plant density, PGR and their interactions were analysed statistically with Genstat version 11.0 using a two-way ANOVA. All data was checked for normality prior to analysis and, if necessary, transformed. When F probabilities were significant ($P < 0.05$), the least significant difference (l.s.d.) was calculated at $P = 0.05$ for testing differences between means.

5.3 Results

Plant establishment, crop growth, yield and alkaloid content were high due to optimal crop management and a well-structured soil type. Very strong winds (106 km/hr; BOM, 2009) were recorded on 22 January 2009. The only significant interaction between sowing rate and PGR treatments occurred with lodging scores recorded at flowering and the main effects of other variables are discussed below.

5.3.1 The effect of sowing rate and Sunny application on plant growth

Plant establishment increased with sowing rate, with close to a 4-fold difference in establishment from the lowest to highest sowing rate (Table 5.1). However, at maturity, with increased plant mortality there was no difference in plant density between the two highest sowing rates and there were more under-developed plants with increasing sowing rate (Table 5.1). PGR treatment had no effect on final plant density. There were also no differences in light interception between treatments (data not presented).

Table 5.1. The effect of sowing rate on plant density, Cressy 2008-09.

Treatment	Mean plant density (plants/m ²)		
	Establishment	Maturity	Maturity -small plants*
Sowing rate (g/ha)			
400	30.2	30.9	0.1
800	58.3	45.9	0.9
1200	83.5	69.9	1.5
1600	116.1	75.6	2.9
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i>0.03</i>
<i>l.s.d._{0.05}</i>	<i>16.98</i>	<i>8.88</i>	<i>1.86</i>

* counts of small plants lower than height of main canopy

Both Sunny treatments resulted in a significant decrease in height of the main stem and primary branch compared with the control (Table 5.2). The split application was more effective than the single application in reducing main stem and primary branch height and this was the only treatment to reduce the height of secondary and tertiary branches. There was no effect of sowing rate on branch height except at the primary inflorescence level ($P = 0.05$) where branch heights for the 400 and 800 g/ha rates (111.1 and 110.1 cm, respectively) were higher than the 1600 g/ha rate (105.7 cm).

Table 5.2. The effect of Sunny application on main stem and branch height at harvest, Cressy 2008-09.

Treatment	Mean height (cm) of branch level			
	Main stem	Primary	Secondary	Tertiary
PGR				
Control	109.9	116.5	116.5	112.3
1L/ha -single	102.5	111.4	113.0	108.8
1L/ha -split	94.9	99.4	101.9	94.1
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>
<i>l.s.d.</i> _{0.05}	3.6	3.46	4.74	7.26

To further examine the different responses in branch height, percentage differences between each main stem and respective branches were compared. At the higher sowing rates, the relative height of the main stem compared with the primary and mean of branches, respectively, were the same ($P > 0.05$) and close to 100% whereas the main stem at the lowest sowing rate was nearly 9% shorter than the primary branch (Table 5.3). There was no effect of sowing rate on percentage difference in height between main stem and secondary branches. For the PGR treatment, height of the main stem and branches for control and split Sunny application were similar, but the single application was significantly shorter i.e. there was a greater difference between the height of the main stem and branches (Table 5.3).

Table 5.3. The effect of sowing rate and Sunny application on the relative difference in height between main stem and branches (as a percentage) at harvest, Cressy 2008-09. There was no significant interaction ($P > 0.05$) between sowing rate and Sunny application.

Treatment	Mean percentage difference in height		
	Main stem to primary	Main stem to secondary	Main stem to mean of branches
Sowing rate (g/ha)			
400	91.2	91.5	91.5
800	94.2	95.0	94.3
1200	94.9	92.4	94.5
1600	97.5	89.8	97.4
<i>F Prob</i>	<i><0.001</i>	<i>0.12</i>	<i>0.01</i>
<i>l.s.d.</i> _{0.05}	<i>2.90</i>	<i>ns</i>	<i>3.10</i>
PGR			
Control	95.6	94.6	95.8
1L/ha -single	92.6	89.1	92.3
1L/ha -split	95.2	92.9	95.2
<i>F Prob</i>	<i>0.04</i>	<i>0.02</i>	<i>0.03</i>
<i>l.s.d.</i> _{0.05}	<i>2.50</i>	<i>3.70</i>	<i>2.69</i>

Higher sowing rates generally accelerated plant maturity. At the start of flowering, plant colour score (i.e. paler green) increased concurrently with sowing rate indicating more advanced plant maturity (Table 5.4). The two highest sowing rates also had fewer flowers remaining open at mid-late flowering compared with the lower sowing rates. Additionally, there were fewer green capsules at the stripey stage for the highest sowing rate compared with the lowest two sowing rates (Table 5.4). Both Sunny treatments were less advanced at the start of flowering (fewer open flowers and lower plant colour score) compared with the control (Table 5.4). However, by stripey capsule stage, only the split application showed a significant delay in maturity.

Table 5.4. The effect of sowing rate and Sunny application on plant maturity at different growth stages, Cressy 2008-09. There was no significant interaction ($P > 0.05$) between sowing rate and Sunny application.

Treatment	Plant colour -dark green (1) to pale green (4)*	Percentage of flowers remaining open (mid-late flowering)	Percentage of green capsules (stripey stage)
Sowing rate (g/ha)			
400	1.8	58.9	9.6
800	2.2	25.2	8.4
1200	2.5	11.7	5.5
1600	2.8	8.2	4.3
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i>0.01</i>
<i>l.s.d._{0.05}</i>	<i>0.28</i>	<i>10.90</i>	<i>3.45</i>
PGR			
Control	2.8	9.5	2.4
1L/ha -single	2.0	5.8	3.2
1L/ha -split	2.2	6.1	15.9
<i>F Prob</i>	<i><0.001</i>	<i>0.05</i>	<i><0.001</i>
<i>l.s.d._{0.05}</i>	<i>0.24</i>	<i>3.24</i>	<i>3.04</i>

*Scored for plant colour at start of flowering.

There was a significant interaction between sowing rate and PGR treatment for both lodging scores taken during flowering (Figure 5.2). With vigorous growth, root lodging commenced at mid flowering but only in the control plots at the higher (1200 and 1600 g/ha) sowing rates. At mid-late flowering, lodging was also evident for the higher sowing rates treated with a single Sunny application. By harvest, lodging varied in severity throughout the trial and was particularly evident at higher sowing rates. Across all sowing rates, plots that received the single Sunny application showed a significantly higher incidence of lodging than the split or control plots (lodging index means of 4.7, 19.0 and 11.9 respectively for split and single Sunny applications and control; l.s.d. = 4.0).

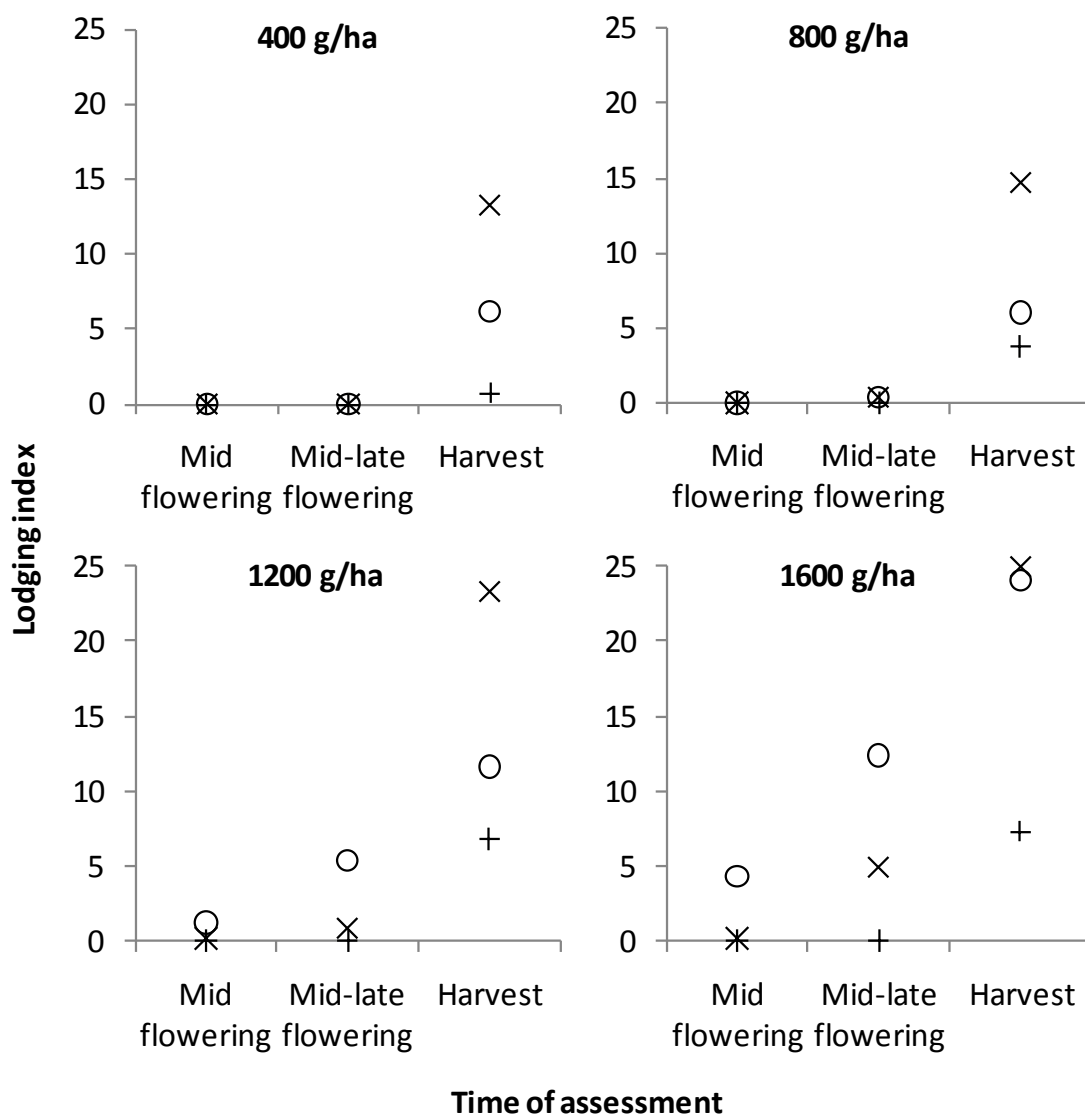


Figure 5.2. The effect of sowing rates (400, 800, 1200, 1600 g/ha) and Sunny application {control (O), 1L/ha -single (X) and 1L/ha -split (+)} on lodging at mid flowering, mid-late flowering and harvest, Cressy 2008-09. l.s.d. = 0.99 for mid flowering, 3.46 for mid-late flowering; non significant interaction at harvest.

5.3.2 The effect of sowing rate and Sunny application on yield components

The split Sunny application increased capsule yield and tended ($P = 0.09$) to produce higher straw yield compared with the control treatment (Table 5.5). Seed yield was greater for both PGR treatments compared with the control and consequently the (S/C)

ratio was significantly lower (Table 5.5). Sowing rate did not significantly affect capsule (358 g/m²), straw (158 g/m²) or seed (200 g/m²) yield.

Table 5.5 The effect of Sunny application on yield and yield components, Cressy 2008-09.

Treatment	Yield (g/m ²)			
	Capsule	Straw	Seed	Straw/capsule ratio
PGR				
Control	325.9	155.1	170.8	0.477
1L/ha -single	356.5	150.1	206.4	0.421
1L/ha -split	391.2	167.3	223.9	0.429
<i>F Prob</i>	<i>0.01</i>	<i>0.09</i>	<i><0.001</i>	<i><0.001</i>
<i>l.s.d._{0.05}</i>	<i>39.46</i>	<i>ns</i>	<i>24.21</i>	<i>0.010</i>

Sowing rate had a significant effect on capsule components. A high sowing rate increased capsules/m² but reduced capsules/plant, capsule weight/plant, individual capsule weight (Table 5.6), number of seeds/capsule and seeds/plant (Table 5.7). As sowing rate had no significant effect on 1000 seed weight, there was also a significant decrease in seed weight/capsule and seed weight/plant with increased sowing rate (Table 5.7).

Table 5.6. The effect of sowing rate and Sunny application on capsule components, Cressy 2008-09. There was no significant interaction ($P > 0.05$) between sowing rate and Sunny application.

Treatment	Capsule components			
	Capsule wt (g)	Capsules /m ²	Capsules /plant	Capsule weight /plant (g)
Sowing rate (g/ha)				
400	0.494	77.4	2.43	12.73
800	0.436	84.2	1.96	8.54
1200	0.386	88.8	1.36	5.04
1600	0.340	98.2	1.30	4.86
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>
<i>l.s.d.</i> _{0.05}	<i>0.0354</i>	<i>9.38</i>	<i>0.369</i>	<i>2.145</i>
PGR				
Control	0.412	80.7	1.74	7.48
1L/ha -single	0.419	84.8	1.62	7.17
1L/ha -split	0.411	96.0	1.93	8.71
<i>F Prob</i>	<i>0.86</i>	<i>0.002</i>	<i>0.15</i>	<i>0.22</i>
<i>l.s.d.</i> _{0.05}	<i>ns</i>	<i>8.12</i>	<i>ns</i>	<i>ns</i>

The split application of Sunny PGR increased the number of capsules/m². There were significant increases in the number of primary, second and tertiary capsules/plant with split applications of Sunny (capsule counts from the six plant samples, data not presented). Sunny application had no significant effect on individual capsule weight and capsule weight/plant (Table 5.6). Seeds/capsule and seeds/m² were significantly higher for both Sunny treatments compared with control plots (Table 5.7) and seeds/plant tended to be higher for the split application ($P = 0.07$). Application of Sunny reduced 1000 seed weight with the split application resulting in a further decrease compared with the single application (Table 5.7). However despite this weight reduction, seed weight/capsule was significantly higher for both Sunny treatments than the control (Table 5.7).

Table 5.7 The effect of sowing rate and Sunny application on seed components, Cressy 2008-09. There was no significant interaction ($P > 0.05$) between sowing rate and Sunny application.

Treatment	Seed components					
	1000 seed wt (g)	Number seeds/m ²	Number seeds /capsule	Number seeds /plant	Seed weight /capsule (g)	Seed weight /plant (g)
Sowing rate (g/ha)						
400	0.500	431486	5583	13543	2.79	6.74
800	0.496	415120	4902	9615	2.43	4.76
1200	0.495	385719	4331	5660	2.14	2.80
1600	0.489	395231	3986	5524	1.94	2.67
<i>F Prob</i>	<i>0.404</i>	<i>0.365</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>
<i>l.s.d.</i> _{0.05}	<i>ns</i>	<i>ns</i>	<i>399</i>	<i>2157</i>	<i>0.220</i>	<i>1.081</i>
PGR						
Control	0.518	330322	4158	7603	2.16	3.94
1L/ha -single	0.490	421152	5012	8380	2.46	4.12
1L/ha -split	0.476	469193	4932	9773	2.36	4.66
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i><0.001</i>	<i>0.07</i>	<i>0.01</i>	<i>0.28</i>
<i>l.s.d.</i> _{0.05}	<i>0.011</i>	<i>48793</i>	<i>346</i>	<i>ns</i>	<i>0.191</i>	<i>ns</i>

5.3.3 The effect of sowing rate and Sunny application on alkaloid content

Increased sowing rate reduced morphine and total alkaloid content. As there was no significant effect on codeine content the proportion of morphine (M ratio) decreased with increased sowing rate (Table 5.8). There were increases in total alkaloid and morphine content with both Sunny treatments (Table 5.8). As the single application increased morphine and reduced codeine content compared with the control, the proportion of morphine was higher.

Table 5.8 The effect of sowing rate and Sunny application on alkaloid content, Cressy 2008-09. There was no significant interaction ($P > 0.05$) between sowing rate and Sunny application.

Treatment	Alkaloid content (%)			
	Total alkaloid	Morphine	Codeine	M ratio
Sowing rate (g/ha)				
400	2.74	2.45	0.115	89.2
800	2.62	2.30	0.123	88.0
1200	2.47	2.18	0.118	87.7
1600	2.42	2.11	0.116	87.4
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i>0.24</i>	<i>0.04</i>
<i>l.s.d.</i> _{0.05}	<i>0.106</i>	<i>0.099</i>	<i>ns</i>	<i>1.29</i>
PGR				
Control	2.34	2.04	0.118	87.2
1L/ha -single	2.69	2.39	0.111	89.1
1L/ha -split	2.66	2.34	0.123	88.0
<i>F Prob</i>	<i><0.001</i>	<i><0.001</i>	<i>0.01</i>	<i>0.01</i>
<i>l.s.d.</i> _{0.05}	<i>0.092</i>	<i>0.09</i>	<i>0.007</i>	<i>1.11</i>

5.4 Discussion

Optimal nutrient, water and pesticide crop inputs on a well structured soil type resulted in high crop yields, 30 % greater than current mean capsule yields of 2.5 t/ha (PACB, 2010). As a consequence, additional plant growth compensated for low plant density and thus there were no interactions between sowing rate and PGR treatments for yield components or alkaloid parameters. A similar lack of interaction has also been recorded in poppies between plant density and irrigation treatments (Chung 1992), suggesting poppy plants under optimal growing conditions are able to compensate for low sowing rates. Main effects of sowing rate and Sunny treatment are thus discussed separately. The optimal plant growth at low plant density also resulted in no significant differences in light interception between treatments.

5.4.1 The effect of sowing rate on growth, yield and alkaloid

There were no significant effects of sowing rate on the height of main stems or branches except for a shorter primary branch with higher plant density. The influence of density on plant height in poppies has not been previously reported, however reduced plant height with greater plant densities in the absence of lodging has been similarly observed in canola (Pahkala *et al.*, 1994). In contrast, higher plant density and increased competition for light has resulted in stem elongation and increased plant height in lupins (Shield *et al.*, 1996), forage turnip (Bilgili *et al.*, 2003) and faba-beans (Dean and Mendham, 2003). In poppies, growth and development of the main stem is significantly more advanced and ceases before that of the branches. When maximum height of the main stem was attained after flowering, it is likely that there was little competition for light between stems even at the higher sowing rates and thus little effect of sowing rate on main stem height. With subsequent growth of lateral inflorescences at closer proximity, there will be increased intra-plant competition for light resulting in greater stem elongation than previously occurred with the main stem. With more branches at the lower sowing rate, it was probably inter-branch competition that resulted in a taller primary branch. Lupins generally have a similar indeterminate growth habit (Noffsinger *et al.*, 2000) and less initial internal competition is also suggested to result in higher pod set (Herbert, 1979) and seeds/pod (Dean and Mendham, 2004) on the main stem inflorescence compared with the later developing lateral inflorescences.

Increased competition between plants at the higher plant densities also resulted in greater plant mortality, more small plants and reduced capsules/plant, individual capsule weight and seeds/capsule. However, with the additional main stems at the high sowing rate, there was an increase in the number of capsules/m² and the net result, on an area basis, was no significant difference in capsule, straw or seed yield between density treatments. These results are comparable to earlier studies with poppies where plant survival was reduced to 66% at high plant densities (Chung, 1990), a decrease from 2.3 to 1.0 capsules/plant at 25 and 200 plants/m² respectively (Chung, 1992) and either small or no differences in capsule yield (Chung, 1990; 1992). High yield plasticity and compensation by yield components at low sowing rates is commonly reported in other field crops e.g. wheat (Whaley *et al.*, 2000), canola (Angadi *et al.*, 2003) and lupins (Lopez-Bellido *et al.*, 2000) and is a result of enhanced radiation-use efficiency and more efficient partitioning of assimilates (Whaley *et al.*, 2000). As in previous plant density studies with wheat (e.g. Holliday, 1960), the stability in capsule yield/m² suggests a ceiling in yield potential above which limitations in site resources result in no further increases. This ceiling also accounts for the lack of significant effect of sowing rate on 1000 seed weight. As seed weight is the last yield component to be determined during crop development (Chung, 1982) and if capsule yield and seed number per unit area have been previously balanced, seed weight should not vary. With very high sowing rates, decreased yield through poor efficiency in utilisation of resources and high levels of disease and lodging have commonly been recorded (e.g. Easson *et al.*, 1993). In poppies, depletion of soil N reduced capsule yield substantially at 200 plants/m² compared with lower plant densities (Laughlin, 1987). It is expected that negative effects on yield at high plant densities will become more evident where site yield potential is lower or restricted by abiotic or biotic constraints.

There was a greater incidence of lodging at the higher sowing rates and this is consistent with earlier studies in poppies with similar and higher plant densities (Laughlin, 1987; Chung 1990; 1992). Increased plant height results in a higher centre of gravity, greater over-turning forces and is a major determinant of lodging (Wiersma *et al.*, 1986; Berry *et al.*, 2000). However plant height was not reported in previous work and in the current study, sowing rate had little effect on maximum height. While not measured, higher sowing rates have been reported to reduce stem diameter in other studies with poppies (Laughlin, 1987), wheat (Easson *et al.*, 1993, Berry *et al.*, 2000) and brassica crops

(Pahkala *et al.*, 1994; Bilgili *et al.*, 2003) leading to earlier onset and/or increased severity of crop lodging. Alternatively, root mass as a proportion of total dry matter was reduced with increasing plant density in poppies (Chung, 1990) and wheat (Easson *et al.*, 1993) and this is likely to decrease plant stability and increase susceptibility to lodging. Lodging response to sowing rate also varied with PGR treatment in the current study and will be discussed in the next section.

Increased sowing rate reduced total alkaloid and morphine content and this is consistent with earlier studies where morphine content at high plant densities was decreased by 8-9% (Laughlin, 1987; Chung, 1990). To maximise alkaloid production, a long finish to the season is required with adequate soil moisture and nutrition (Fist 2001). Higher sowing rates in the current study accelerated maturity and thus reduced the duration of alkaloid biosynthesis and total alkaloid content. Morphine is the final alkaloid in the morphinan pathway (Dewick, 2002; Hagel and Facchini, 2010) and is consequently more affected by early maturity as reflected in the decreased proportion of morphine. Consistent with this, morphine content in morphine poppies is decreased with earlier flowering induced by both water deficit and extended photoperiod (Mahdavi-Damghani *et al.*, 2010) and increases with delayed leaf senescence through supplemental irrigation (Chung, 1987). Alkaloid concentration may also be decreased through plant lodging. As in cereals (Pinthus, 1973), it is likely that lodging would impact on translocation of carbohydrates in the poppy plant and potentially restrict alkaloid latex vessels. For example, sclerotinia (*Sclerotinia sclerotiorum*) infection influences the distribution of morphine in stems, leaves and capsules and was suggested to disrupt normal translocation of morphine through latex vessels (Laughlin and Munro, 1983). Adequate radiation is also required to maximise alkaloid production (Bernath and Tetenyi, 1981) and the greater lodging at the higher plant densities is likely to have resulted in lower light intensity and thus reduced alkaloid percentage. As with impacts on grain yield (Batch, 1981; Fischer and Stapper, 1987), the timing of crop lodging will be important as early lodging is likely to have a far greater impact on alkaloid biosynthesis than later lodging.

5.4.2 The effect of Sunny application on growth, yield and alkaloid

Application of Sunny resulted in a significant decrease in plant height and this is consistent with the PGR screening in Chapter 3 and other trials conducted by GlaxoSmithKline and Tasmanian Alkaloids researchers (unpublished). Earlier studies with PGRs applied to poppies have also shown similar reductions in plant height. In a field trial in Tasmania, daminozide applied at the rosette stage was the most effective PGR in reducing plant height (Forbes and Laughlin, 1985). A chlormequat /ethephon mix and paclobutrazol applied at the start of capsule development reduced stem height (Nagy *et al.*, 1989) and the latter PGR also decreased height in ornamental poppies (*P. nudicaule*) (Martinetti *et al.*, 1994). However, effects from the two Sunny treatments varied; multiple applications reduced height for all branches whereas the single application only decreased height of the main stem and primary branch. As there is little alkaloid in the stems of poppy plants, commercial harvest operations remove only plant capsules and minimal stem (Laughlin, 1987; Fist, 2001). Consequently, a large range in capsule heights after a single application of Sunny is likely to result in some main stem capsules remaining after harvest. The loss in efficacy of PGRs has been previously documented and may result from increased flux of GA precursors after PGR application and these precursors being available after PGR inhibitive effects decline (Rajala, 2004; Poole, 2005). In cereals, the compensation effect can be even more pronounced, particularly where there is sufficient soil moisture to allow further growth (Poole, 2005). Whereas cereal PGRs are applied in late winter, Sunny in this trial was applied in late spring and with warmer temperatures, there is likely to be more rapid metabolism of the PGR. Compensatory growth could also be expected to be particularly strong when conditions for further growth are enhanced such as under irrigation.

Over the duration of flowering, both Sunny treatments at the highest sowing rate reduced the incidence of lodging. While there have been a number of studies where PGR application has reduced plant height in poppies, there have been little data published on effects on lodging due to trials either being conducted in controlled environments or at field sites with conditions not conducive to lodging. Only Forbes and Laughlin (1985) in a field trial in Tasmania have reported decreased lodging in poppies after application of PGRs, with daminozide the most effective. While it is likely that the decreased lodging in Sunny treated plots was due to reduced plant height and

lower centre of gravity, PGRs may also reduce lodging by thickening of plant stems. Application of PGRs has increased stem diameter in wheat (Batch, 1981; Crook and Ennos, 1995; Tripathi *et al.*, 2003) and rice (Kim *et al.*, 2007). Stem diameter has also been increased in poppies with application of a chlormequat/ethephon mix and paclobutrazol (Nagy *et al.*, 1989). In the current study, stem diameter was not measured but stems were noticeably more rigid with less movement in the wind. As stem diameter is often reduced with higher plant density, the potential benefit of PGRs in increasing stem diameter may account for the reduced lodging at higher sowing rates with Sunny treatment compared with control plots. PGRs may also reduce lodging through decreased incidence of stem disease and for some, such as eyespot (*Pseudocercospora herpotrichoides*) in wheat, this is probably through increased stem diameter and reduced damage to stems (Pinthus, 1973). Many of the triazole class of PGRs are also recognised as plant fungicides with different enantiomers displaying specificity for inhibition of GA or sterol biosynthesis in fungi (Rademacher, 2000). Sclerotinia (*S. sclerotiorum*) increases susceptibility to crop lodging (Dennis, 1998; Laughlin *et al.*, 1998) and has been reported at higher densities in canola (Davies *et al.*, 1999). However, in the current study, three fungicides were applied and no disease was observed.

The single application of Sunny tended to have less effect on lodging than the split application but notably, after flowering, lodging was generally more severe than in control plots. It was likely lodging occurred with strong winds (106 km/hr) on 22 January 2009 (BOM, 2009). There may be several explanations for this increased lodging. In measurements on stem strength (Dean, unpublished), the internodes near the top of the poppy plant were more springy and flexible and not inclined to snap under force. In cereals, the compensation effect after application of PGR increases the length of the upper internodes in particular (Waddington and Cartwright, 1986). Lodging has been noted to start with initial crop leaning (Crook and Ennos, 1995). Potentially longer upper internodes with the single application of Sunny may have caused greater leaning of branches resulting in higher over-turning forces and increased lodging. The greater relative height differences between the main stem and branches for the single application compared with the control and split application of Sunny may also have influenced the incidence of lodging. It was observed that poppy capsules are blown by the wind at slightly different frequencies and consequently not all capsules on the same

plant are travelling in the same direction at the same time. In this way, capsules may act as counter-balances for other capsules and reduce the lodging forces. The stem of the main inflorescence is visually thicker and with a heavier capsule (Chung 1987) acts as the largest counter-balance. Thus with a relatively short main stem from a single application of Sunny, there is likely to have been less counter-balance effect from this capsule resulting in more lodging compared with the control and split application.

The relative proportions of capsule yield components at the time of lodging may also influence lodging more than total capsule weight/plant. While the single application of Sunny had no effect on mature capsule weight, the seed weight/capsule was greater and the straw to capsule ratio was reduced compared with the control treatment. Maximum growth rate of seeds in the capsule occurs after that of the capsule wall (Chung, 1982) and by maturity is generally over 50% of the capsule weight. If there is differential drying between seeds and the capsule wall with seed drying more slowly, the single Sunny application capsules with a higher proportion of seed would have been heavier than the other PGR treatments prior to maturity. A higher centre of gravity would thus result in greater potential for lodging. This could be tested simply by picking poppies at different growth stages, drying and then measuring the relative weight of seed and capsule wall.

The split application of Sunny increased capsule yield largely through greater capsules/m² and also tended to increase capsules/plant. Comparable increases in branching and capsules/plant have been achieved with application of the PGR triacontanol (Srivastava and Sharma, 1990; Khan *et al.*, 2007) and chlormequat applied to ornamental poppies (*P. nudicaule*) also increased the number of flowers (Martinetti *et al.*, 1994). Increased seed yield with the split application of Sunny was partly attributable to more capsules but also through elevated seed weight/capsule. This occurred despite a reduction in 1000 seed weight as both Sunny treatments resulted in a large increase in seeds/capsule. Stem elongation is a phase of rapid growth when the stem is a strong sink, diverting assimilates away from the developing capsules and seeds. In Chapter 3, it was postulated that stem shortening with application of Sunny leads to further partitioning of assimilates to seed. It appears this is largely through additional capsules but concurrent with the flush of assimilates, more seeds are initiated or realised in each capsule. Although not always consistent, increases in grain yield

from cereal crops with plant application of anti-GA PGRs are well documented (e.g. Tolbert, 1960; Humphries *et al.*, 1967; Knapp *et al.*, 1987; Rajala and Peltonen-Sainio, 2002). Consequently an increase in seed yield in poppies is not unexpected, but the large increase in seed number has not been shown previously. There was no difference in seeds/capsule between the Sunny treatments but the increase in capsules/plant with the split application resulted in greater intra-plant competition and 1000 seed weight was reduced compared with the single Sunny and control treatments.

The split application of Sunny also tended to increase straw and seed yields compared with the single application and this is consistent with results from an earlier trial conducted by Dean (2005-06, unpublished). The effect of multiple doses is probably cumulative; with each application, there is further partitioning of assimilates from the stem into capsule wall and seeds through more capsules/plant and capsules/m². Multiple PGR applications have been applied to reduce vegetative growth in cereals after the PGR is metabolised, particularly when applied at early stem elongation (Waddington and Cartwright, 1986; Poole 2005) but there have been no reports of increased grain yield. However, in apples, yield has been improved with multiple PGR applications through additional control of vegetative growth (Spinelli *et al.*, 2010). Interestingly, split applications of Moddus (Chapter 4) had a similar additive effect but through increased thebaine content.

Application of Sunny delayed maturity and this is consistent with the PGR screening trials (Chapter 3). Delayed flowering with application of paclobutrazol has also been reported in ornamental poppies (*P. nudicaule*) (Martinetti *et al.*, 1994) and in canola (Armstrong and Nicol, 1991). In cereals there has been little effect of PGRs on flowering date (Tripathi *et al.*, 2004; Toyota, *et al.*, 2010); however a number of studies have reported delayed senescence (Tolbert, 1960) in particular of the flag leaf (Naylor, 1989) peduncle and ear (Lowe and Carter, 1970). Application of triazole PGRs has also increased cytokinin levels and decreased ABA and ethylene levels, all effects contributing to delayed senescence (Grossman *et al.*, 1991; Grossman, 1992). As discussed in Chapter 2, triazole PGRs also inhibit ethylene biosynthesis and this may result in enhanced flux of S-adenosyl methionine. This molecule is also a precursor for biosynthesis of cytokinin but in addition spermidine and spermine, which at higher levels also delay tissue senescence (Grossman, 1992). In the current study, both Sunny

treatments were significantly greener at early flowering. Application of triazole PGRs has been observed to produce greener leaves in other studies (Armstrong and Nicol, 1991; Grossman, 1992; Fletcher *et al.*, 2000) and where measured, has increased chlorophyll content (Grossman *et al.*, 1991; Baldwin *et al.*, 2009; Kang *et al.*, 2010). As a decrease in the size of individual leaves has been recorded after PGR application (Green *et al.*, 1985; Kang *et al.*, 2010), enhanced green leaves may be a result of increased chlorophyll content per unit leaf area (Fletcher *et al.*, 2000).

Application of both Sunny treatments increased the mean total alkaloid and morphine content by 14 and 16% respectively. There have been a number of reports of higher morphine yields in poppies following application with PGRs. Application of triacontanol significantly increased morphine content both applied solely (Srivastava and Sharma, 1990) and in combination with foliar applications of GA₃ (Khan *et al.*, 2007). Results with ethephon have been variable and depending on rate and timing, both reduced and increased the concentration of alkaloids in the capsule (Ramanathan, 1978; Ramanathan, 1981; Hsu and Forman, 1982). However, these trials were generally conducted under controlled conditions and/or morphine content measured from lanced rather than dry mature capsules as in the current study. Secondary metabolite content has also been enhanced after application of PGRs in other species. GA and pyrethrin biosynthesis share a common precursor and increased terpene and thus pyrethrin levels in *Chrysanthemum cinerariaefolium* have been attributed to increased metabolic flux from inhibition of GA biosynthesis (Haque *et al.*, 2007). Application of CCC has also increased levels of artemisinin in *Artemisia annua* (Shukla *et al.*, 1992) possibly through a similar mechanism.

The increased total alkaloid with application of Sunny is comparable with results from the PGR screening trials in Chapter 3 where it was suggested that this may be due to stem shortening leading to reduction in competing sinks for plant assimilates and increased metabolite flux for alkaloid biosynthesis. However, it could be expected that the additional reduction in plant height with split applications would further increase alkaloid content and this did not occur. Alternatively, by delaying maturity, application of Sunny extends the duration of alkaloid biosynthesis resulting in increased total alkaloid content. This explanation is also consistent with accelerated maturity and decreased alkaloid with the higher sowing rates discussed previously. In Chapter 3 and

the current study, alkaloid profile was also altered after application of Sunny with a higher proportion of the “final” alkaloid in the respective pathways: oripavine in thebaine poppies and morphine in morphine poppies, both effects that could be expected with delayed maturity.

Extended duration of alkaloid biosynthesis may also account for differences in alkaloid profile with timing of applied Sunny. The single application of Sunny at late rosette tended to increase the proportion of morphine compared with the split application. This is consistent with a previous study where there was higher morphine and lower codeine content with a late rosette application compared with applications at mid stem elongation and hook stages (Dean, unpublished). Thus the earlier application appears to have greater efficacy in increasing morphine content and by splitting the rate, so that only one-third of the chemical was applied at the most sensitive growth stage, the effectiveness of the split rate application was reduced.

CHAPTER 6. GENERAL CONCLUSIONS AND RECOMMENDATIONS

With demand for greater area, poppy production in Tasmania has moved to soil types more prone to restricted root growth from water-logging. Raised bed farming systems alleviate winter water-logging enabling crops to be sown in winter and early spring. However, earlier sowing often results in excess vegetative growth that commonly leads to crop lodging, harvest losses and lower alkaloid content. PGRs are used in a number of crops to reduce excess vegetative development and can also modify yield and quality. The effects of a range of PGRs used commercially in agricultural and horticultural crops were evaluated on poppy plant growth and alkaloid yield. Responses were often variable but this is not surprising given the complex interactions between different plant hormones, growth stage and environmental stimuli. Several effects were however consistent and of significant economic benefit for the poppy industry.

Plant growth and height were reduced by several PGRs but application of Sunny produced the most consistent effects. Single applications of Sunny however increased lodging and this may be due to the greater height differential between main stem and lateral capsules, reduced counter-balance effect from main stem capsules and/or greater bending of upper internodes. The height difference between main stem and branch capsules with a single dose is also likely to result in either poor harvestability or alkaloid dilution. Although there was little effect of Moddus on plant height, the incidence of lodging appeared to be reduced in these and additional trials (A. Fist pers. comm.). Further work is required to evaluate the mode of action of both Sunny and Moddus in reducing crop lodging and, in particular, to determine whether there are effects on root systems and stem diameter as can occur in other crops.

Seed yield was the component most affected by PGR application. As thebaine residues limit the commercial value of thebaine poppy seed, the consistent and large effect of Slow Grow in reducing seed weight is of potential benefit to industry. In contrast, application of Sunny increased capsule and seed yield due to a large increase in seeds/capsule. It is proposed that Sunny-induced reduction in plant height leads to partitioning of assimilates to yield components. A similar effect may also occur with

application of Moddus but as seed yield decreased and straw yield tended to increase, reallocation is instead from seed tissue to capsule wall. Splitting Moddus and Sunny applications resulted in additional effects over that of single applications. Effects from multiple doses appear to be cumulative; with each application, there was additional partitioning of assimilates from the stem to seeds or from seeds to capsule wall for Sunny and Moddus respectively.

The most marked effect of PGRs on alkaloid content was a consistent increase in thebaine and reduction in oripavine content with application of Moddus. As total alkaloid did not vary with single applications, it was postulated that Moddus inhibits the formation of oripavine from thebaine. Recently, it has been confirmed that a 2-oxoglutarate-dependent dioxygenase catalyses the demethylation of thebaine to oripavine and the activity of this same enzyme is known to be inhibited by Moddus in GA biosynthesis (Hagel and Facchini, 2010). Whereas responses to rate and timing of Moddus application were small and inconsistent, multiple and split applications tended to further increase thebaine content, again suggesting a cumulative dose effect.

Application of Sunny resulted in a different alkaloid response to that of Moddus. Total alkaloid and in particular morphine content were increased and this may be the result of extended biosynthesis of alkaloid through delayed maturity. Alternatively, by decreasing plant height, Sunny may reduce the size of competing sinks for plant assimilates resulting in increased flux of morphinan pathway precursors. A similar effect may also occur with reduced seed yield following multiple applications of Moddus and reallocation of assimilates to alkaloid production. It would be useful to examine the effect of single and multiple applications of Sunny and Moddus on partitioning of assimilates within the plant with carbon labelling techniques.

Given the trends of increased alkaloid yield with split and multiple applications of Moddus and Sunny, further trials are required to validate these benefits and to determine optimum rates at different growth stages. From a practical consideration, multiple or split applications are commercially feasible. The economic value of poppies justifies the cost of extra applications of PGRs, given that commercial poppy crops are already sprayed with fungicide every 10-12 days from early stem elongation to the end of flowering. Combinations of PGRs may also be effective, particularly as Moddus

appears to have a consistent action on alkaloid profile with effects not altered by other PGRs. The results from this study suggest that an effective strategy may be to apply Slow Grow at early stem elongation to reduce seed yield with subsequent Moddus treatment to increase thebaine content. Rates of approximately 3-4L/ha for Slow Grow would appear to be appropriate; however further refinement is required.

From this study, split applications of Sunny are recommended with the first application at late rosette to early stem elongation to reduce main stem height and increase morphine content. Additional applications are likely to increase branching, capsules/plant and yield. Branch height reduction is also necessary to minimise lodging and improve crop harvestability. The benefits of decreased lodging and increased alkaloid yield with application of Sunny have also demonstrated the long term merit in breeding shorter stature poppies as has successfully occurred with other crops. Despite increased capsule yield, as application of Sunny tended to decrease the thebaine ratio this may restrict Sunny to application in morphine poppy crops but this requires further evaluation.

The results from this study have shown significant economic benefits can be obtained with application of PGRs to poppy crops. Effects of Sunny treatment on poppy growth and yield are consistent with trials previously conducted by industry but this study helps explain the mechanisms of alkaloid and seed yield increases. Sunny is now marketed as Sumagic and applied to many commercial poppy crops in Tasmania. With the ability to control excess vegetative growth, there is increased confidence in earlier sowing of crops and the potential for higher yields. Application of Moddus resulted in large increases in thebaine content and combined with a tendency to elevate straw yield, consistently increased thebaine yield around 25%. As approximately half of Tasmania's poppy alkaloid production is now derived from thebaine poppies, this is economically a very significant result for the industry. Results from this study have been confirmed in industry trials and has led to registration of Moddus in poppy crops and close to 100% adoption rates by thebaine poppy growers (A. Fisk pers. comm.).

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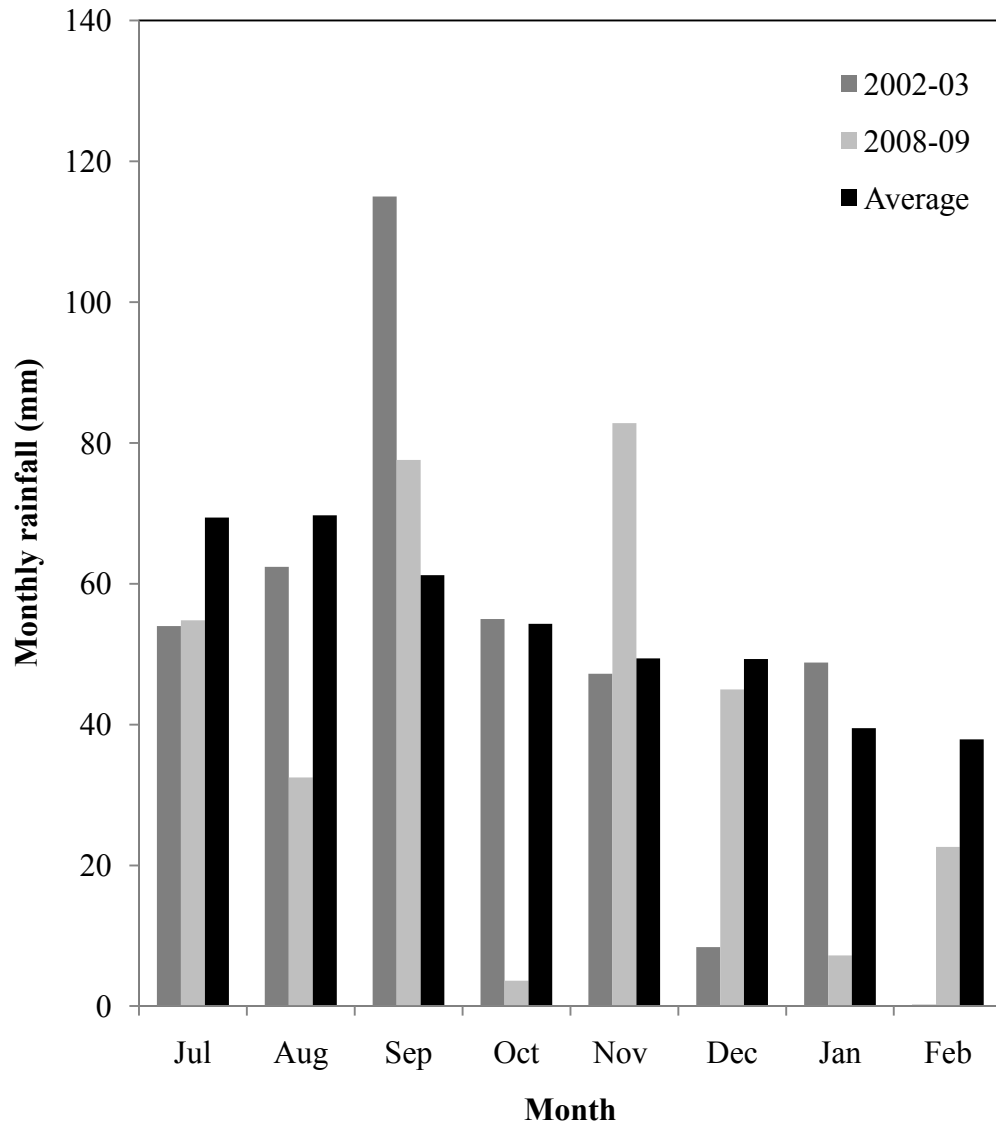
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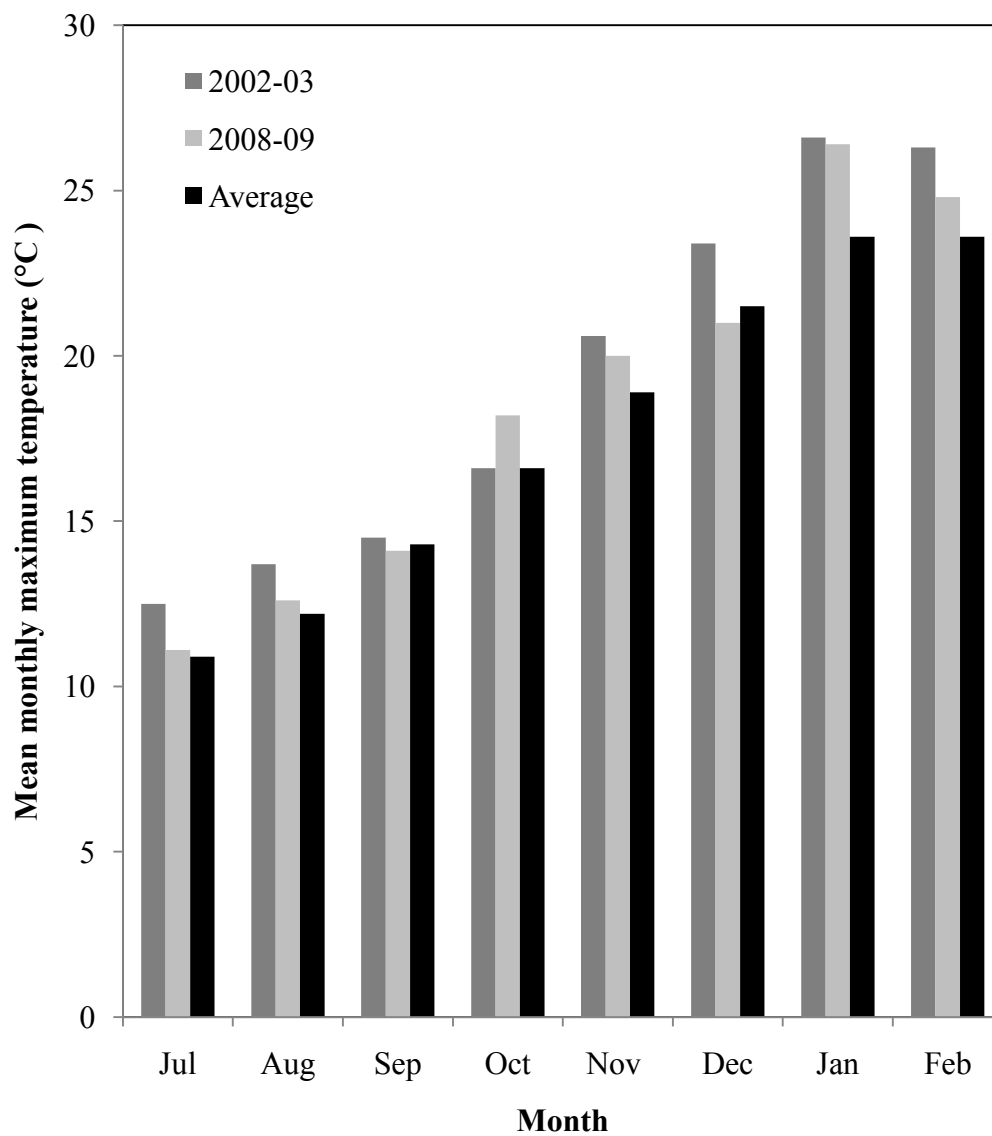
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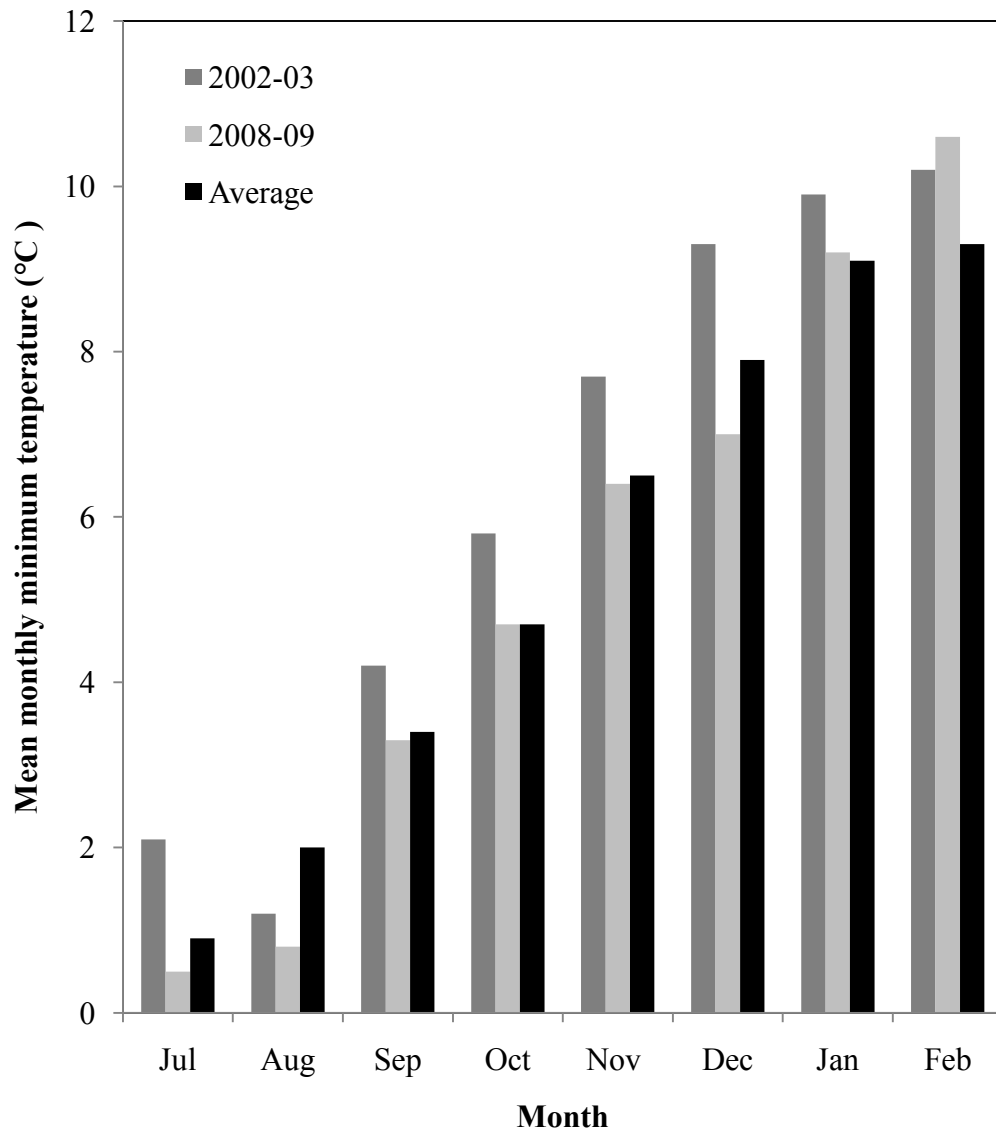
APPENDIX 1



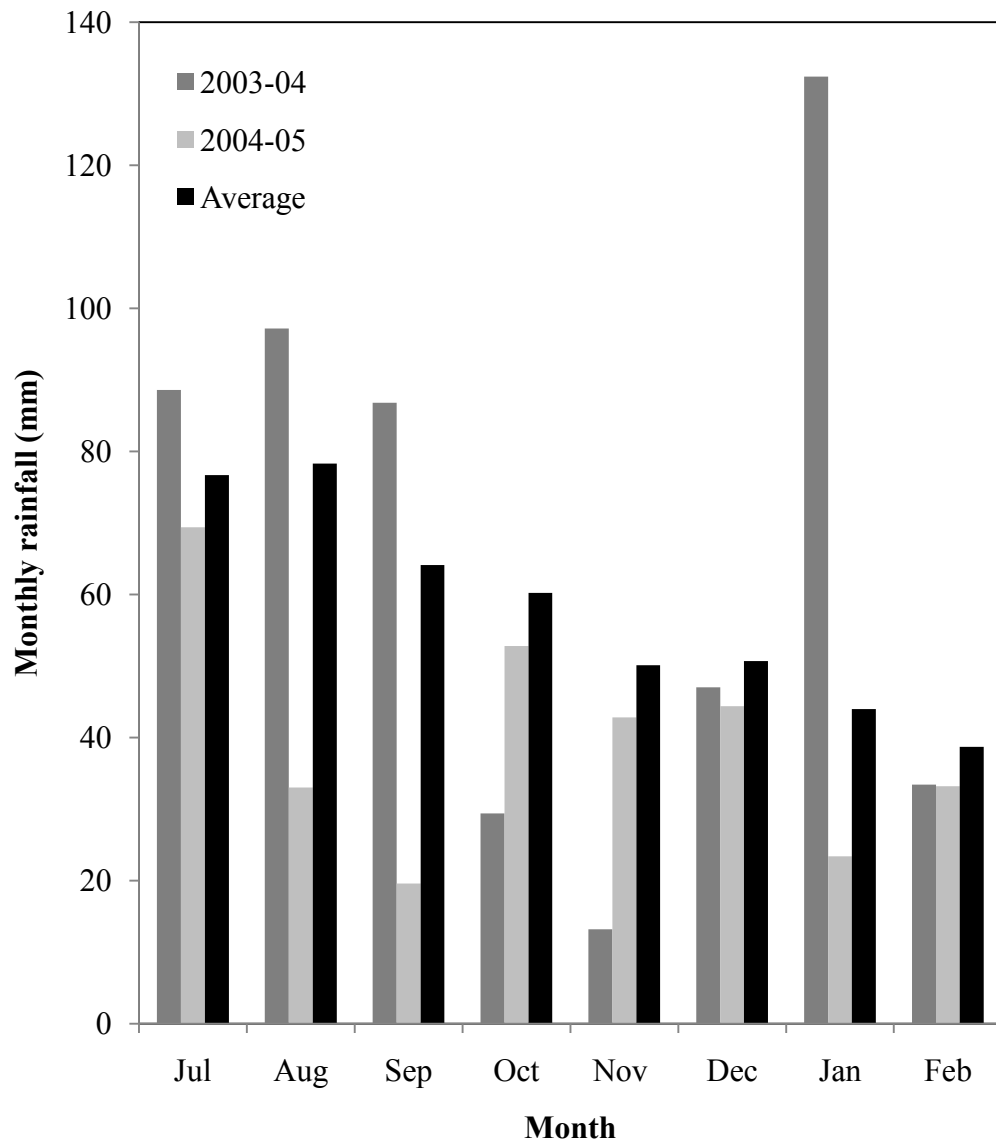
Appendix 1(a). Monthly rainfall at Cressy (147.08°E, 41.72°S) during the study period of 2002-03 and 2008-09 relative to the long term average monthly rainfall.



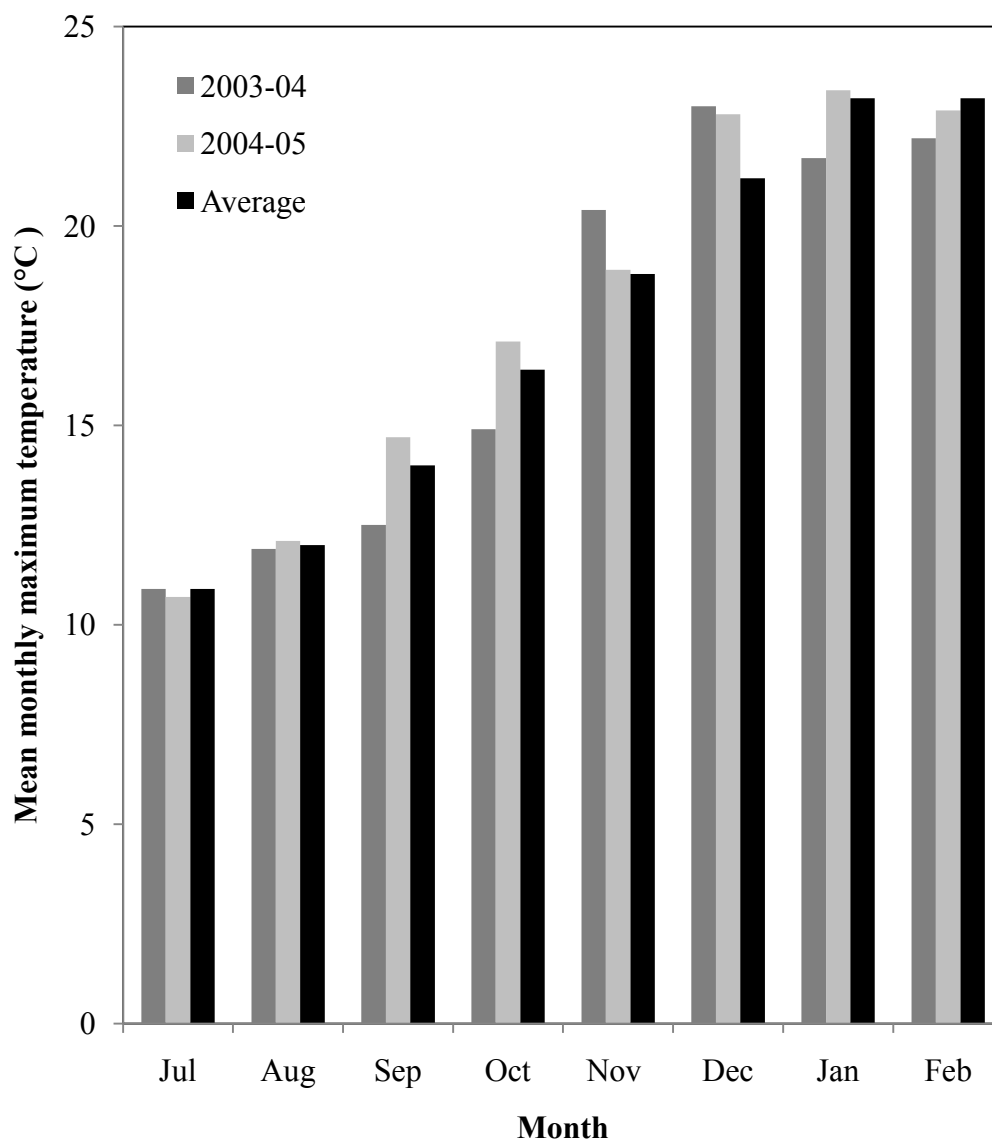
Appendix 1(b). Mean monthly maximum temperature measured at Cressy (147.08°E, 41.72°S) during the study period of 2002-03 and 2008-09 relative to the long term average.



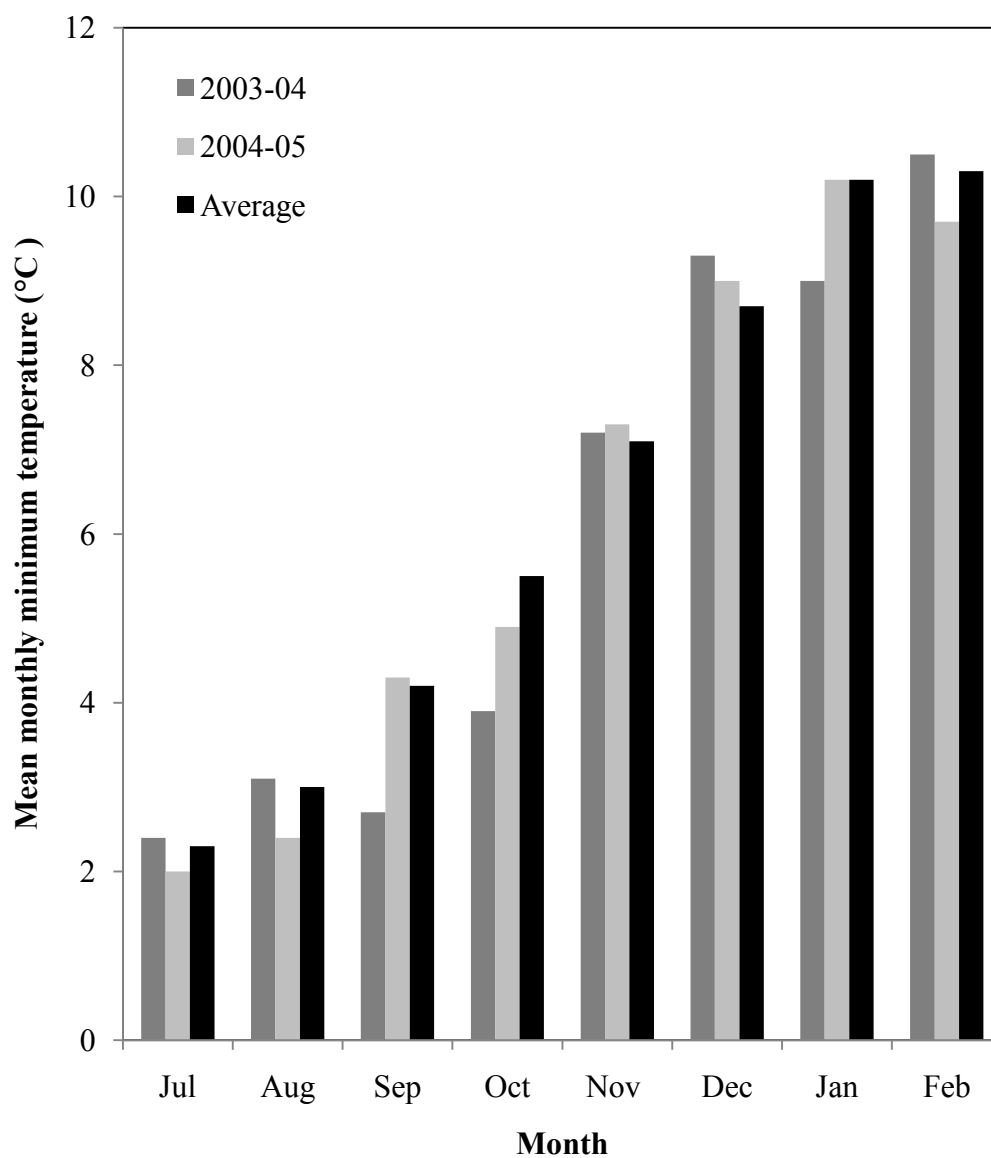
Appendix 1(c). Mean monthly minimum temperature measured at Cressy (147.08°E, 41.72°S) during the study period of 2002-03 and 2008-09 relative to the long term average.



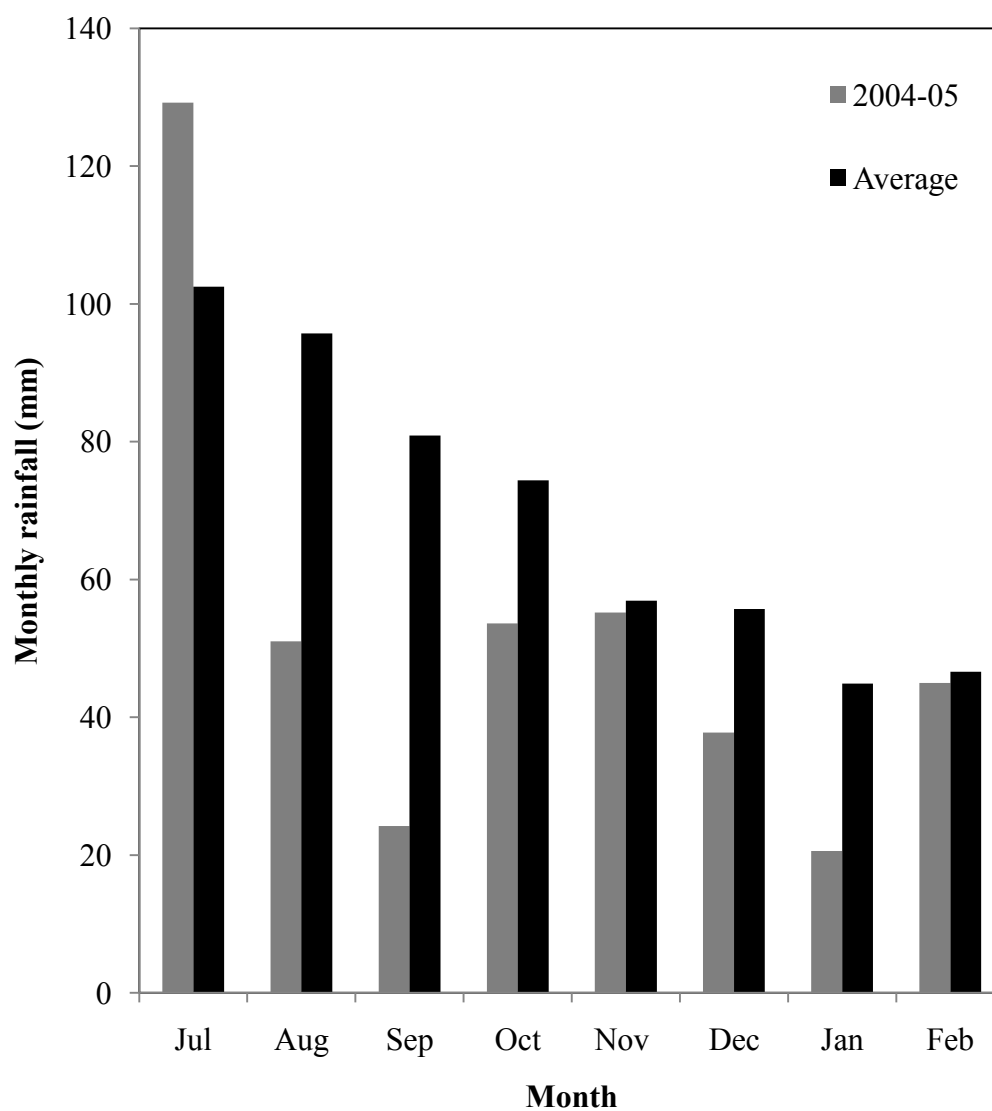
Appendix 1(d). Monthly rainfall at Launceston Airport (147.21°E, 41.55°S) during the study period of 2003-04 and 2004-05 relative to the long term average monthly rainfall.



Appendix 1(e). Mean monthly maximum temperature measured at Launceston Airport (147.08°E, 41.72°S) during the study period of 2003-04 and 2004-05 relative to the long term average.



Appendix 1(f). Mean monthly minimum temperature measured at Launceston Airport (147.21°E, 41.55°S) during the study period of 2003-04 and 2004-05 relative to the long term average.



Appendix 1(g). Monthly rainfall at Westbury (146.81°E, 41.52°S) during the study period of 2004-05 relative to the long term average monthly rainfall.