Effect of cytokinin and gibberellin on potato tuber dormancy

by

Jan Adriaan Rossouw

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Magister Scientiae (Agric.): Agronomy

Department of Plant Production and Soil Science

In the Faculty of Natural and Agricultural Sciences
University of Pretoria

Pretoria

Supervisor: Prof. P.S. Hammes

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DECLARATION

I, Jan Adriaan Rossouw, hereby declare that this thesis for the degree M Sc (Agric): Agronomy at the University of Pretoria is my own work and has never been submitted by myself or any other University. The research work reported is the result of my own investigation, except where acknowledged.

J.A. Rossouw

January 2008

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ABSTRACT

The effect of cytokinin and gibberellin, and in particular a combination of the two, on termination of dormant potato tubers was investigated. The objective was to effectively terminate dormancy through the external application of a combination of cytokinin and gibberellin. Freshly harvested tubers were treated and either cut at the stolon end with the apical portions placed on moist cotton wool, or left intact and dry. Tuber segments treated with a high concentration of cytokinin ($0.1g.L^{-1}$) or a combination of cytokinin and gibberellin sprouted within 5 days, whereas high gibberellin concentrations ($0.1g.L^{-1}$) stimulated sprouting within 9 days. Untreated tuber segments supplied only with moisture terminated dormancy later than hormonal treated tubers, but much earlier than segments that were kept dry.

Tuber segments treated with a combination of cytokinin and gibberellin, or a high concentration of gibberellin ($0.1g.L^{-1}$), produced more and longer sprouts than tubers treated with only cytokinin ($0.1g.L^{-1}$) or a low concentration of gibberellin ($0.005g.L^{-1}$).

Sprouts on tuber segments treated with a combination of cytokinin and gibberellin attained maximum sprout growth rate nine days after treatment, but thereafter the growth

rate decreased. This decrease may be a consequence of closed plasmodesmata although membrane permeability and its affect on assimilate availability may play a role.
This phenomenon deserves further research attention. Removal of wound periderm did not reactivate sprout growth.

The wounding of tubers by removing a portion at the stolon end and supplying moisture greatly enhanced the termination of dormancy and subsequent sprout growth, indicating that the availability of water may be a factor in initiation of sprouts.

The results are compatible with the hypothesis that cells in dormant buds are arrested in the G1 phase of the cell cycle. Cytokinin is needed to initiate cell cycling, but gibberellin is also needed to initiate and maintain cell growth. These two growth regulators are also involved in the opening of the plasmodesmata as well as the creation of new plasmodesmata witch would establish communication between the apical meristem and the rest of the tuber.

Keywords: *Solanum tuberosum,* cytokinin, gibberellin, cell cycle, sprout growth, plasmodesmata, growth rate

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CHAPTER 1
GENERAL INTRODUCTION

Potato is the fourth most important crop in the world. In South Africa potatoes constitutes over 40% of the commodities sold on the fresh produce markets (Niederwieser, 2003; Department of Agriculture, 2003).

Potato seed constitutes about 13% of the total potato production in South Africa (http://www.potatoes.co.za; Niederwieser, 2003). The seed certification scheme specifies tolerance levels for pests and diseases, and seed must be certified before it is dispatched to farmers. Seed tubers can only be tested for certain virus diseases once dormancy has been terminated and the tubers are sprouting.

Tubers have a natural dormant period. By definition a structure is dormant when no visible growth is observed because of endogenous or environmental factors (Hemberg, 1985; Burton, 1989). Potato tubers will only sprout once endogenous factors permits it to grow and environmental factors are favorable. In some cultivars it can take months before dormancy is terminated.

To date no method has been developed to effectively terminate dormancy, though a large range of products and treatments have been shown to affect dormancy and sprout growth. In South Africa gibberellins are registered to stimulate sprouting. Recent research focused on the use of ethanol to terminate dormancy (Claassens *et al,* 2005; Vreugdenhil *et al*, 2006) but it is at best as effective as gibberellins. A substance (aminoethoxyvinylglycine and 1-methylcyclopropene) claimed to terminate dormancy, has recently been patented in the U.S.A (Hansen *et al*, 2004). Rindite has been used to terminate dormancy, but its toxicity hampers its use (Nasiruddin & Blake, 1997; Kim *et al*, 1999). Bromoethane have also been reported to terminate dormancy (Coleman & Coleman, 1986; Coleman *et al*, 1992) as well as ethylene (Rylski, Rappaport & Pratt, 1974; Suttle, 1998a) and elevated levels of carbon dioxide (Coleman & McInerney, 1997; Coleman, 1998; Singh & Ezekiel, 2005). These

methods are either not completely effective, or as in the case of Rindite, may be toxic to humans and the environment.

Dormancy termination is important in speeding up the process of virus testing and seed certification. Much research has been done on this topic, but the exact mechanism of dormancy is still to be established. A lot of progress has been made especially on biotechnological aspects, but the role of plant growth regulators in dormancy initiation and termination is not yet clear. It has been found that plant growth regulators and in particular abscisic acid, gibberellins and cytokinins play an important role in regulating potato tuber dormancy. Gibberellins and cytokinins have a stimulatory effect in terminating tuber dormancy.

The objective of this thesis is to investigate the effect of cytokinin and gibberellin on termination of dormancy and on sprout growth of fresh potato tubers.

CHAPTER 2

THE PHYSIOLOGICAL BASIS OF DORMANCY

2.1: INTRODUCTION

Bud dormancy is a characteristic prevalent in many plant species. It can be initiated by various factors, including moisture stress, high or low temperatures, day length and heredity (Hartmann *et al,* 2002). There is no universally accepted definition of dormancy. Hemberg (1985) defines dormancy as the collective stage where a bud will not sprout because of endogenous or exogenous conditions. He refers to the phase of endogenous dormancy as the rest period, but it is also defined as innate or deep dormancy. Burton (1989) defined a bud as being dormant when there was no visible growth because of endogenous or exogenous factors.

Crabbé & Barnola (1996) divide dormancy into three categories, namely endodormancy; paradormancy and ecodormancy. Hilhorst & Toorop (1997) suggested that dormancy be divided into two categories, namely primary or innate dormancy and secondary or induced dormancy. In seed dormancy, primary dormancy is imposed during the development of the seed and secondary dormancy occurs when the environmental conditions are not favorable. The same terminology can be used in bud dormancy. Buds have a natural or primary dormancy period in which they will not sprout even if conditions are favorable for sprouting, but sprouting can also be inhibited if environmental conditions are not favorable. This is termed eco- or secondary dormancy. Hartmann *et al.* (2002) also suggested that dormancy be divided into two main categories, namely primary and secondary dormancy.

Endodormancy is dependent on the genetic characteristics of the plant. It is determined by cultivar as well as the growing and storage conditions (Hemberg, 1985; Burton, 1989), and termination of dormancy will only take place after a period of rest. Dormancy dictated by exogenous factors, including temperature, day length and humidity, can be terminated by altering the conditions so as to make it more favorable (Van Der Schoot, 1996).

Fresh potato tubers are in a state of endogenous dormancy which must be terminated before sprout growth will commence.

2.2: THE ROLE AND MECHANISM OF DORMANCY

Plants have adapted to environmental conditions through evolutionary processes. One of the strategies developed to survive extreme conditions is dormancy. Dormancy, more specific endodormancy, is part of a plants' genetic design. The potato tuber is a modified stem (Coleman, 1987) that has developed an endodormant phase to protect it against conditions in which the plant will otherwise not survive. Burton (1989) suggested that the duration of dormancy should be calculated from the time the tubers are initiated until sprouting commences.

A dormant bud is active though no visible growth is observed (Burton, 1989), and DNA, RNA as well as proteins are synthesized by the resting organ (Suttle, 1996), but the process is much slower than in actively growing organs (Van der Schoot, 1996). Coleman (1987) stated that the process of growth is ongoing during dormancy and though there is no visible growth, the buds remain physiologically active.

It is not clear how dormancy is initiated, but it is believed that plant growth regulators play an important role in the initiation and termination of dormancy. The first stage of dormancy takes place when a tuber is initiated. In this stage tubers act as sink organs and the buds are dormant.

Suttle (1996) stated that the nuclei of dormant buds are predominantly (77%) in the G1 phase, with only about 13% in the G2 phase. This is in agreement with the notion that growth is not terminated in a dormant bud, but that it is ongoing, although much slower than in an actively growing bud.

In cell division four phases can be distinguished, namely the G1; S; G2 and M phases (Figure 2.2.1). The G1 phase occurs between mitosis (M-phase) and DNA-synthesis (S-phase) and

the G2 phase between DNA-synthesis and mitosis (Fairbanks & Anderson, 1999). A G0 phase also exists where non-cycling cells are arrested. During the G-phases the cells prepare for the next phase of the cell cycle. Between the G1-S and G2-M phases are checkpoints of cyclin-dependent protein kinase (Cdk). Cdk must be bound to a protein, called cyclin, for the cycle to commence through the checkpoint to the next phase of cell division (Francis & Sorrell, 2001).

Suttle (1996) mentions that especially one family of regulatory proteins, the P-34 kinases of which Cdc 2 kinase is the most important, is actively involved in cell division. These proteins are regulated by cyclins. In plants D-cyclins are necessary for G1 to S regulation. The D-cyclins can react on external signals. It has been found that cytokinins are able to induce D-cyclins to bind to Cdk proteins and initiate the G1 to S phase (Francis & Sorrell, 2001).

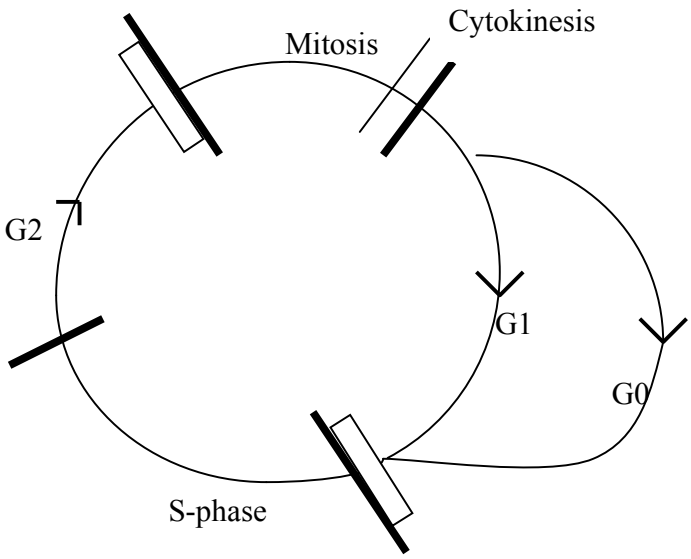

Figure 2.2.1: The cell cycle illustrating different phases and checkpoints (Francis & Sorrell, 2001)

It is possible for cytokinins to stimulate non-cycling cells in the G0 phase to start division and act in the G1-S phase. Sucrose may be involved in the G1 to S transition by inducing CycD2, and the G1 to S phase may be blocked if an energy source is not available (Francis & Sorrel, 2001). Based on the available literature it is postulated that dormancy is mainly

regulated by the cell cycle and that plant growth regulators play an important part in regulating dormancy.

2.3 THE ROLE OF PLASMODESMATA IN DORMANCY

Dormancy is primarily regulated by the cell cycle, but the plasmodesmata (PD) may play an important role in maintaining the dormant state. Plant cells are protected by a cell wall. Within cell walls small connecting 'tubes', plasmodesmata, exist that connect adjacent cells. The plasmodesmata are responsible for cell-to-cell communication (Roberts, 2005). Primary plasmodesmata (Figure 2.3.1) form during cytokinesis. During plasmodesmal formation the endoplasmic reticulum become trapped between the developing cell walls. The plasma membrane surrounds the endoplasmic reticulum, forming a sleeve around the endoplasmic reticulum, compressing it to form a tube, called a desmotubule (Roberts, 2005). Secondary plasmodesmata forms after cytokinesis and can develop across any cell wall to increase communication between cells (Zambryski & Crawford, 2000). Most of the transport takes place through the cytoplasmic sleeve (Van der Schoot, 1996; Zambryski & Crawford, 2000; Roberts, 2005).

During dormancy the plasmodesmata is connected within the apical meristem (Van der Schoot, 1996) but the connection between the surrounding cells are temporarily blocked by callose formation at the entry points (Zambryski & Crawford, 2000). During the early part of dormancy no endoplasmic reticulum exists in the plasmodesmata (Van der Schoot, 1996). The lack of endoplasmic reticulum may be one of the main causes for the regulation of dormancy because no cell-to-cell communication can take place between meristimatic cells and surrounding cells.

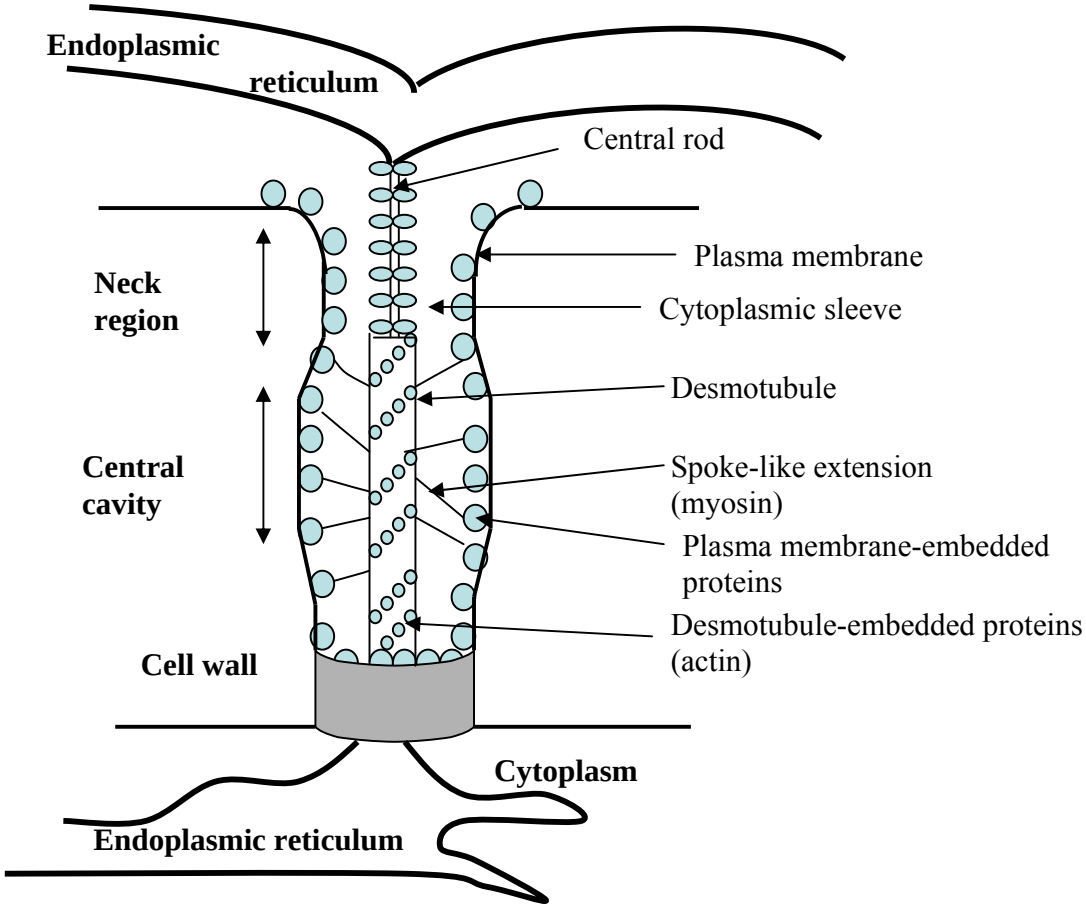

Figure 2.3.1: Diagram depicting open plasmodesmata with helically arranged actin proteins on the desmotubule and myosin spokes connecting the actin to the plasma membrane (Roberts, 2005)

The apical meristem of tubers is normally the first to break dormancy. The apical meristem consists of two layers, namely the tunica and corpus (Van der Schoot, 1996; Rinne & Van der Schoot, 2003; Rinne & Van der Schoot 2004). The tunica and corpus cells differ in their growth pattern as well as physiology and genetic lay-out (Rinne & Van der Schoot, 2004). Signaling transport between tunica and corpus cells cannot take place through diffusion but only through the plasmodesmata (Rinne, Kaikuranta & Van der Schoot, 2001). Because the apical meristem is isolated from neighboring cells, cold signals must act directly on the bud (Rinne & Van der Schoot, 2003).

During dormancy callose deposits are formed at the neck of the plasmodesmata. These deposits are also referred to as sphincters and consist of 1,3-β-D-glucan (Rinne *et al*, 2001). Callose can also be deposited as a reaction to wounding or pathogen attack to stop the loss of assimilates out of plant cells and the invasion of pathogens into the cells (Roberts, 2005).

Besides the formation of callose, an actin-myosin mechanism as well as a calcium-binding cytoskeletal protein, centrin, may be involved in regulating flow through the plasmodesmata (Rinne & Van der Schoot, 2004; Roberts, 2005). The centrin protein and actin-myosin regulate flow inside the plasmodesmata whereas callose blocks flow outside the plasmodesmata. Deposits of 1,3-β-D-glucan swells when in contact with water and seal the plasmodesmata (Rinne & Van der Schoot, 2003). According to Rinne & Van der Schoot (2003, 2004) the 1,3-β-D-glucan serves as an external sphincter as well as an internal plug during dormancy. As long as the plasmodesmata stays blocked, no cell to cell communication can take place. Rinne *et al.* (2001) showed that gibberellins injected into dormant cells does not move to other cells within six hours after injection and they postulated that it could be because of the 1,3-β-D-glucan restrictions. They hypothesized that it may take longer than six hours for gibberellins to move through blocked plasmodesmata.

During the reactivation of the apical meristem, spherosomes (small translucent vacuoles) appear near the cell membrane and plasmodesmata. The enzyme, 1,3-β-D-glucanase is responsible for the breakdown of 1,3-β-D-glucan and is present in the spherosomes. Abscisic acid is involved in the formation of spherosomes and may have an important role in dormancy regulation by preparing the cell for dormancy release (Rinne *et al*, 2001; Rinne & Van der Schoot, 2003, 2004). Rinne & Van der Schoot (2004) observed that during a cold period the spherosomes were displaced and in contact with the plasmodesmata in porous tree species like birch, populus as well as in potato tubers.

Pétel & Gendraud (1996) demonstrated that the cells underlying a bud in dormant Jerusalem artichoke (*Helianthus tuberosus* L.) tubers and peach tree cuttings act as a nutrient sink. Sprouting will only commence when this sink becomes a source for the meristem. According to Crabbé & Barnola (1996) the parenchyma of Jerusalem artichoke tubers act as a strong

sink and that dormancy will only be broken if sink strength is reversed between parenchyma and the meristem. During the transition from sink to source the plasmodesmata becomes highly branched (Zambryski & Crawford, 2000; Schulz, 2005, Roberts, 2005). Zambryski & Crawford (2000) hypothesized that this may restrict or diminish transport of assimilates, but according to Schulz (2005) this phenomenon happens to stop any backflow of assimilates. He hypothesized that because the plasmodesmata can not widen, it branches to increase transport. This is directly contradicting the hypothesis of Zambryski & Crawford (2000).

Crabbé & Barnola (1996) suggested that endodormancy is enforced by a permeability barrier between the parenchyma and apical meristem. This barrier consists of a pH gradient between the bud and the surrounding cells. With this pH gradient intact, little or no nutrients and hormones are transported to the apical meristem and the tuber stay dormant.

Currently little information exist on the effect of plasmodesmata on cytokinin mobilization and it may be interesting to find out what effect cytokinin has on the formation of 1,3-β-D-glucan and dormancy. Ormenese, Bernier & Pèrilleux (2006) reported that cytokinins are able to promote secondary plasmodesmata in mustard (*Sinapsis alba*) but the effect of cytokinins on tuber plasmodesmata is still not clear.

2.4 EFFECT OF PLANT GROWTH REGULATORS ON DORMANCY

Dormancy is regulated by mainly three plant growth regulators, namely abscisic acid (ABA), gibberellin (GA) and cytokinin. All of these plant growth regulators are produced via the mevalonic acid pathway (Figure 2.4.1).

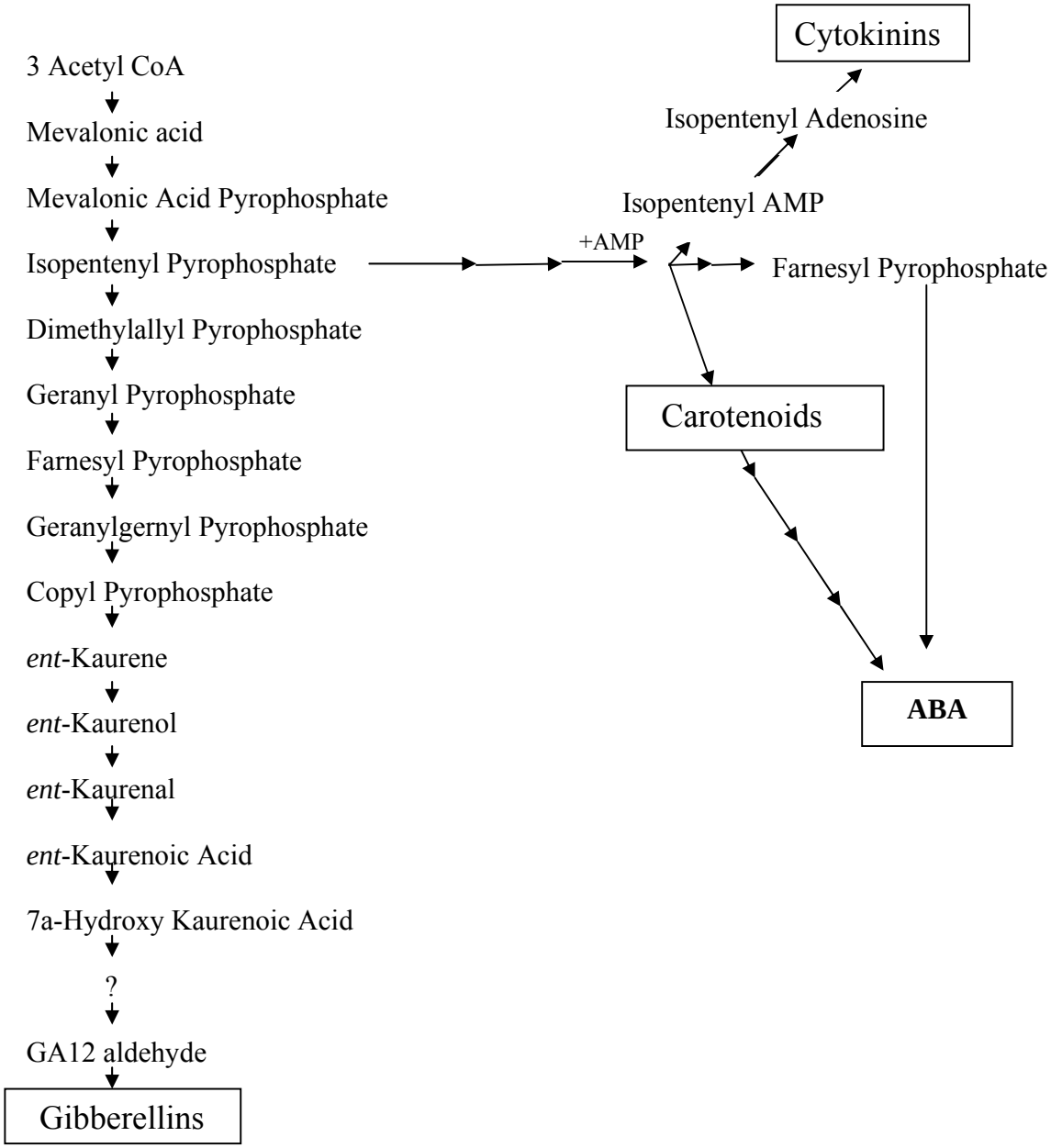

Figure 2.4.1: Mevalonic acid pathway with the different end-products (Arteca, 1996)

2.4.1: Abscisic acid

Abscisic acid (ABA) is a naturally occurring growth inhibitor present in all organs of higher plants. Its function is concentration dependent (Hartmann *et al*, 2002). ABA (a terpenoid) is produced in the chloroplasts and other plastids (Gardner, Pearce & Mitchell, 1985; Arteca, 1996). ABA acts on various processes in plants, like stomatal opening and closure, abscission, cold stress, and dormancy (Arteca, 1996).

Transport of ABA takes place throughout the plant (Gardner *et al*, 1985) and inactivation can take place in a similar way as that of gibberellins and cytokinins (Arteca, 1996). ABA has been found to play a central role in dormancy regulation. Potato tubers have developed a dormant phase vital in surviving extreme cold winter conditions. ABA plays a pivotal role in the protection against cold stress (Arteca, 1996) and it has been proven that shorter days trigger the production of ABA (Gardner *et al*, 1985). Various authors agree that the level of ABA is highest in freshly harvested tubers and that the level decline during storage (Coleman, 1987; Suttle & Hulstrand, 1994; Arteca, 1996; Suttle, 1996; Biemelt *et al*, 2000; Claassens & Vreugdenhil, 2000; Fernie & Willmitzer, 2001). Leclerc *et al.* (1995) stated that small microtubers had a higher ABA content than field grown tubers and that the higher content was the reason for the longer dormant period.

It has been suggested that it is only the initial level of ABA that is important in triggering dormancy (Hilhorst & Toorop, 1997; Biemelt *et al,* 2000), but Coleman (1987) and Suttle (2004a) are of the opinion that ABA is also important in maintaining dormancy. According to Hill (1980) and Gardner *et al.* (1985) it is not necessarily the level of ABA, but the ratio of ABA to gibberellins that is the regulatory factor in maintaining dormancy. If the ratio is in favor of gibberellins, sprouting will commence and dormancy will be terminated (Hill, 1980). ABA is required to initiate dormancy, but there is not a definite level below which ABA must decrease for sprouting to commence (Claassens & Vreugdenhil, 2000).

Hemberg (1985) suggested that ABA is involved in the inhibition of DNA and RNA synthesis and that gibberellins are involved in the acceleration of DNA and RNA synthesis.

ABA is produced to protect the tuber against cold damage and, through inhibition of DNA and RNA synthesis will arrest the cell in the G1 phase of the cell cycle until the GA: ABA ratio is in favor of gibberellins to promote cell division and sprouting. Most authors agree that exogenously applied ABA will inhibit sprouting (Hemberg, 1985; Burton, 1989; Suttle, 1996; Suttle, 2004a) but that it is concentration dependent (Suttle, 2004a).

2.4.2: Gibberellins

Gibberellins are growth promoters. To date, over a hundred gibberellins have been isolated (Vivanco & Flores, 2000), but not all are active in plants. Gibberellins in the early 13-hydroxylation group, especially GA $_1$ and GA $_4$ are the most active (Suttle, 1996; Vivanco & Flores, 2000; Suttle 2004a; Suttle, 2004b). Increased activity is probably due to the lactone ring present in the structure of these gibberellins (Gardner *et al*, 1985).

Gibberellins are also synthesized through the mevalonic acid pathway (Figure 2.4.1). Rajala & Peltonen-Sainio (2000) as well as Arteca (1996) mentioned that all plants use the same pathway to produce gibberellins up to the GA$_{12}$-aldehyde phase from where different gibberellins are then synthesized. Gibberellins are mainly produced in the leaves but may also be synthesized in the roots and fruit (Gardner *et al*, 1985; Vivanco & Flores, 2000). Transport takes place mainly in the phloem of the plants and can be both up- and downwards (Kefeli, 1978). Gibberellins are responsible for cell elongation, rather than cell division (Kefeli, 1978; Vivanco & Flores, 2000; Francis & Sorrell, 2001), but may play a role in stimulating cell division in meristimatic areas (Kefeli, 1978; Roberts, 1988).

When gibberellins are applied to dormant tubers, dormancy can be broken according to Hemberg (1985); Coleman (1987); Burton (1989) and Fernie & Willmitzer (2001). Gibberellins may terminate dormancy by activating the synthesis of DNA and RNA (Bruinsma *et al*, 1967; Clegg & Rappaport, 1970; Burton, 1989; Arteca, 1996) and by decreasing the duration of the cells in the G1 and S phases (Roberts, 1988). According to Francis & Sorrell (2001) gibberellins may affect the Cdc 2 kinase level at the G2-M

checkpoint of the cell cycle and gibberellins may increase the rate at which cells are produced.

Barendse (1975) suggested that GA 1, GA 4 and GA 7 are precursors of GA 3. GA 1 is the most biologically active gibberellin (Jones, Horgan & Hall, 1988). Jones *et al.* (1988) indicated that GA 20 is the precursor of GA 1. Xu *et al*, (1998) found that GA 1, GA 4, GA 9 and GA 20 are present in potato plants and that GA 4 and GA 9 levels do not change during tuber development, but only the level of GA 1. These results concur with the suggestion of Jones *et al.* (1988) that GA 1 is biologically the most active gibberellin. Vreugdenhil & Sergeeva (1999) found the same range of gibberellins in the genotype *Solanum demissum*. Suttle (1996) mentioned that exogenously applied GA 12 was metabolized in the shoot apices of potatoes to produce GA 1, GA 8, GA 20, GA 29, GA 44, GA 51 and GA 53.

Suttle (2004b) found that exogenously applied GA 1, GA 19 or GA 20 promoted tuber sprout growth, and that GA 1 was the most active. He observed that endogenous gibberellin content had a stronger effect on the sprout growth than on the termination of dormancy. Burton (1989) suggested that gibberellins are likely to be a controlling factor in the growth of sprouts. Both Hemberg (1985) and Van Ittersum & Scholte (1993) observed that the endogenous gibberellin concentration increased before dormancy was broken. The concentration increases even more once sprouting commenced (Suttle, 1996).

Besides the synthesis of DNA and RNA, gibberellins are also believed to have an effect on the reducing sugar content (Hill, 1980; Mares, 1985). According to Hill (1980) the process of starch breakdown is gibberellin dependent. In seeds gibberellins are responsible for synthesis of enzymes like α-amylase that helps in the breakdown of starch into sugars. Claassens & Vreugdenhil (2000) mentioned that gibberellins in potatoes had a stimulating effect on the reducing sugar content, but it could not be proven whether gibberellins had an effect on starch breakdown. Coleman (1987) reported that gibberellins increased the synthesis of reducing sugars, but only after the storage tissues were no longer dormant.

Tuber wounding also has a stimulatory effect on the synthesis of gibberellins (Shih & Rappaport, 1970). Ewing, McMurry & Ewing (1987) found that wounding had a stimulatory effect on the termination of dormancy, but could not establish what the reason was.

2.4.3: Cytokinins

Cytokinins are synthesized via the mevalonic acid pathway (Figure 2.4.1). All the cytokinins originate from isopentenyladenosine, a substrate found in the mevalonic acid pathway (Arteca, 1996; Vivanco & Flores, 2000). The cytokinins most prevalent in plants are those with a N6-side chain like zeatin, isopentenyladenine and N6-benzyladenine (Vivanco & Flores, 2000; Mok & Mok, 2001). A change in the N6-side chain has pronounced effects on the activity of the cytokinins and the biological activity of the cytokinins may be lost (Mok & Mok, 2001).

The main effect of cytokinins is on cell division (Arteca, 1996; Francis & Sorrell, 2001; Hartmann *et al*, 2002; Vreugdenhil, 2004), but cytokinins also have an effect on cell enlargement (Arteca, 1996). Cytokinins act on the G1-S and the G2-M phases of the cell cycle (Roberts, 1988; Francis & Sorrell, 2001; Mok & Mok, 2001; Suttle, 2004a). In the G1-S transition, cytokinins function by inducing the CycD3 genes (Francis & Sorrell, 2001; Mok & Mok, 2001). Plants over-expressing CycD3 could maintain cell division without exogenously applied cytokinins. Cytokinins have also been found to be active in the G2-M transition of the cell cycle where induction of a histone-H-kinase, cdc2, takes place (Francis & Sorrell, 2001; Mok & Mok, 2001). Cytokinins are also responsible for creating a nutrient sink (Sattelmacher & Marschner, 1978; Hannapel *et al*, 2004) which is essential in maintaining the G1-S and G2-M transitions (Francis & Sorrell 2001).

Hemberg (1985) mentioned that cytokinins applied exogenously can break dormancy of potato tubers, and the levels of endogenous cytokinins increase before the termination of dormancy. More than one form of cytokinin is found in potato tubers. Sattelmacher & Marschner (1978) as well as Van Staden & Dimalla (1977) found that zeatin riboside is the main component of cytokinins in potatoes, but Suttle & Banowetz (2000) stated that *cis*-

zeatin and not *cis*-zeatin riboside, increase in tubers during dormancy and is responsible for the termination of dormancy. The authors also mentioned that isopentenyladenine and *trans*-zeatin levels increase in tubers during storage. Suttle (1998b) found eight different forms of cytokinins present in potato tubers with isopentenyl adenine-9-glucoside the most abundant. The levels of the zeatin type cytokinins were comparable with that of the isopentenyl-type (IP) cytokinins. Isopentenyl adenine-9-glucoside is biologically inactive and serves as a precursor for zeatin-type cytokinins which are biologically active. The IP- type cytokinins must first be synthesized to zeatin-type cytokinins before dormancy can be terminated (Suttle, 1998b).

Endogenous levels of cytokinins must increase before dormancy can be broken (Banaś, Bielińska-Czarnecka & Klocek, 1984; Suttle, 2004a). Increase in cytokinin content coincides with a reduction of acid inhibitors like ABA (Claassens &Vreugdenhil, 2000). Tissue sensitivity to cytokinins is important in the regulation of dormancy (Turnbull & Hanke, 1985) and exogenously applied cytokinins were only effective at certain times in the dormancy period, mostly at the beginning and end of dormancy (Coleman, 1987). Suttle (2001) found that cytokinins were unable to stimulate sprouting directly after harvest but that dormant tubers reacted to an injection of cytokinins at a dose dependent rate. Koda (1982) found that resting tubers, when wounded, exhibited a significant increase in cytokinins, seemingly zeatin glucoside, and that it could have an effect on sprouting.

Based on the available literature it is postulated that cytokinins are essential in the regulation of dormancy, probably acting in synergy with other hormones, especially gibberellins in terminating dormancy.

CHAPTER 3

EFFECT OF CYTOKININ AND GIBBERELLIN ON POTATO TUBER DORMANCY

3.1 ABSTRACT

The effect of cytokinin and gibberellin, and in particular a combination of the two growth hormones, on dormancy termination of potato tubers was examined in three trials. Greenhouse tubers of the cultivars Caren and Up-to-date, as well as three cultivars obtained from a private institution, namely Cult. A, Cult. B and Hermes, were used. Tubers were immersed in solutions of cytokinin (BAP), gibberellin (GA_{4+7}) or a combination of the two. Tubers were cut at the stolon end and the apical portions placed on moist cotton wool in a dark growth chamber at 20˚C (Trial 1 and 3). In Trial 2 tubers were either cut and the apical portions placed on moist cotton wool, or the tubers were left intact and dry. In Trial 1 tubers of cv. Caren exposed to a high concentration of cytokinin (0.1 gL^{-1}), or combinations of cytokinin and gibberellin, started to sprout within 5 days after treatment, whereas high gibberellin treatments (0.1gL^{-1}) stimulated sprouting within 9 days. In Trial 2, hormonal treatments on Cult. A, Cult. B and Hermes showed similar results, but intact tubers sprouted considerably later. In Trial 3 tubers of cv. Up-to-date did not react in a time dependent manner to hormonal treatments, but sprouted relatively early throughout the trial. The study showed that combinations of cytokinin and gibberellin terminate dormancy earlier than treatments of only gibberellin, provided the tubers are cut and placed on a moist medium.

3.2 INTRODUCTION

Virus testing is essential for certification of seed potatoes. Testing for certain viruses, like potato leaf roll virus (PLRV), requires tubers with actively growing sprouts. Some cultivars have a longer dormant period which delays virus testing. Gibberellins stimulate sprouting (Clegg & Rappaport, 1970; Claassens & Vreugdenhil, 2000), but are often not efficient on dormant tubers. Cytokinins have also been reported to break dormancy (Claassens & Vreugdenhil, 2000). Coleman (1987) reported tubers to be sensitive to cytokinin treatment at the beginning and end of dormancy, whereas Suttle (2001) found that cytokinins did not stimulate sprouting of tubers directly after harvest, but tubers did react to cytokinins in a time dependent manner.

Gibberellins function by stimulating and accelerating DNA and RNA synthesis (Clegg & Rappaport, 1970; Shih & Rappaport, 1970; Burton, 1989). Gibberellins are also involved in the synthesis of reducing sugars (Clegg & Rappaport, 1970; Claassens & Vreugdenhil, 2000). This action increases before sprouting commences and the sugars are utilized by the growing sprout. Cytokinins affect the transport of nutrients and hormones (Kefeli, 1980) and create sink regions to attract assimilates (Sattelmacher & Marschner, 1978).

During dormancy, meristems are arrested in the G1 phase of the cell cycle (Crabbé & Barnola, 1996; Suttle, 2004a). Francis & Sorrell (2001) suggested that cytokinins are able to act on the G1 phase and induce G0 cells to enter the cell cycle. By inducing D-cyclins to bind to Cdk proteins, the transition from the G1 to S phases can take place. Cytokinins are also responsible for the transition from the G2 to M phase through the induction of Cdc 2 kinase (Mok & Mok, 2001).

Both cytokinins and gibberellins stimulate sprouting by activating certain phases of the cell cycle. Research has focused mainly on the use of either cytokinins or gibberellins separately, and little information exists on their combined application to terminate dormancy. Hill (1980) reported that cytokinins alter the growth of axillary buds and that applying gibberellins will accelerate bud growth. Kefeli (1978) mentioned that in general,

translocation of cytokinins in plant tissues is weak and that the presence of other hormones will increase the effect of cytokinins, with the maximum effect attained by using a combination of growth hormones. Gardner *et al.* (1985) also mentioned that gibberellins generally act synergistically with cytokinins. Chen (1975) hypothesized that cytokinins may be involved in the disruption of growth inhibitors and in so doing, allow gibberellins an opportunity to function. According to Low (1975) a combination of benzyladenine or kinetin and gibberellic acid stimulated growth in old buds of soybeans.

The objective of this thesis was to find a method to effectively terminate dormancy in potato tubers. It was hypothesized that dormancy could be terminated effectively by using cytokinin and gibberellin in combination. The effect of cytokinin and gibberellin was also evaluated with regard to the time dependent manner in which the dormant buds reacted to applications of these hormones.

3.3 MATERIALS AND METHODS

Tubers of cultivars Caren and Up-to-date were multiplied in greenhouses at the Experimental Farm of the University of Pretoria from March to September 2006. In addition three cultivars were obtained from a private institution, namely Cult. A, Cult. B and Hermes. The cultivars Caren and Up-to-date have a short to medium dormant period of 50 to 70 days whereas Hermes has a long dormant period of approximately 100 days (Niederwieser, 2003). Cult. A also has a relatively short dormant period, whereas Cult. B has a long dormant period.

When tubers reached a size of approximately 20g the haulms were removed ten days before harvest to allow skin set. Harvested tubers were kept in a dark growth chamber at 20˚C and used within two weeks. Tubers were dipped in a 0.6 gL^{-1} dimethyl didecyl ammonium chloride solution (Sporekill) to contain soft rot during experimentation. After drying, the tubers were immersed in the treatment solutions for one hour. The treatments consisted of either gibberellin (GA_{4+7}) or cytokinin (Benzylamino purine (BAP)) or combinations of the two. Tubers were either left intact and dry, or cut at the stolon end and the apical portions placed on moist cotton-wool in plastic containers. All the tubers were placed in a dark growth chamber at 20˚C. Tubers were checked daily for signs of sprouting. A tuber was considered sprouted when a sprout reached a length of 2mm. The average date of sprouting was taken when at least 80% of the tubers had sprouted.

Trial 1

Twenty tubers of cultivar (cv.) Caren were exposed to each of the following treatments:

- Control — Distilled water
- 1 BA — 0.1gL^{-1} cytokinin
- 1GA — 0.1gL^{-1} gibberellin
- 1BA:1GA — 0.1gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- 1BA:2GA — 0.1gL^{-1} cytokinin + 0.2gL^{-1} gibberellin

- 2BA:1GA — 0.2gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- 0.5BA — 0.05gL^{-1} cytokinin
- 0.5GA — 0.05gL^{-1} gibberellin
- 0.5BA:0.5GA — 0.05gL^{-1} cytokinin + 0.05gL^{-1} gibberellin
- 0.5BA:1GA — 0.05gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- 1BA:0.5GA — 0.1gL^{-1} cytokinin + 0.05gL^{-1} gibberellin
- SRCR — 0.005gL^{-1} gibberellin

(Standard registered commercial rate)

Trial 2

The genotypes Hermes, Cult. A and Cult. B were used. The tubers were either cut and placed on moist cotton wool or kept intact and dry. Due to a limited supply of tubers, only eight tubers were used per treatment and only six treatments were applied.

The treatments consisted of the following:

- Control — Distilled water
- 1GA — 0.1gL^{-1} gibberellin
- 1BA:1GA — 0.1gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- 1BA:2GA — 0.1gL^{-1} cytokinin + 0.2gL^{-1} gibberellin
- 2BA:1GA — 0.2gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- SRCR — 0.005gL^{-1} gibberellin

Trial 3

Up-to-date tubers were harvested ten days after haulm removal and stored in a dark room at 20˚C. The first treatments were applied one day after harvesting. Over the following five weeks 10 tubers per treatment were treated every seven days.

The treatments consisted of the following:

- Control — Distilled water
- SRCR — 0.005gL^{-1} gibberellin
- BA:GA — 0.005g^{-1} cytokinin + 0.005gL^{-1} gibberellin

3.4 RESULTS AND DISCUSSION

Trial 1

Intact tubers of cv. Caren remained dormant for at least eight weeks, while the untreated tuber-segments on a moist medium sprouted after 18 days (Figure 3.1). Within five days after treatment 80% of tubers exposed to a high concentration of cytokinin (1BA) or combinations of cytokinin and gibberellin had started to sprout, with the exception of 1BA:0.5GA. Gibberellin application stimulated sprouting after approximately 10 days.

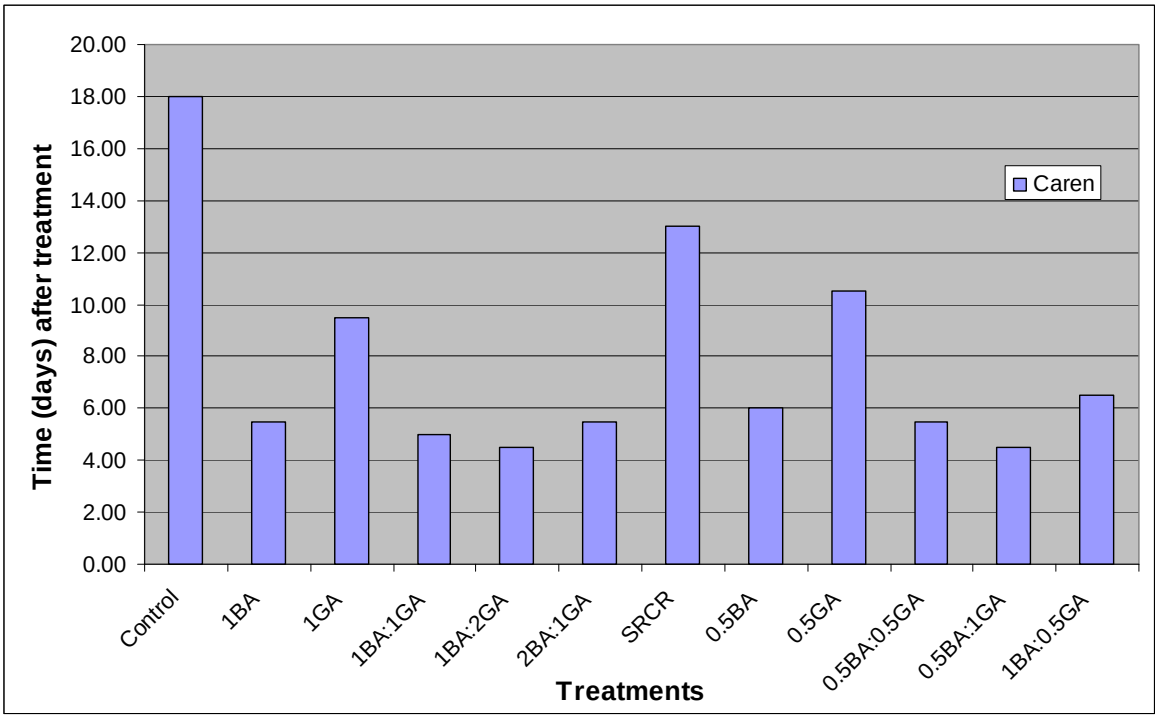

Figure 3.1: Time to 80% dormancy termination of cv. Caren after treatment with cytokinin and gibberellin

Data not statistically analyzed

Only one data set for SRCR

Trial 2

Where tuber segments were placed on a moist medium, all three cultivars reacted to the treatments in a similar way as the cultivar Caren in Trial 1. It took eight days before the control and gibberellin treatment of Cult. A and Hermes sprouted (Figure 3.2). Where a

combination of cytokinin and gibberellin was applied, tubers started sprouting within four days. Sprouting reaction of Cult. B was identical to the other two cultivars, although the control only sprouted after 18 days. Gibberellins did not have an effect on dormancy termination in Cult. A and Hermes. Gibberellin treatments did result in earlier sprouting in Cult. B, which has a long dormant period.

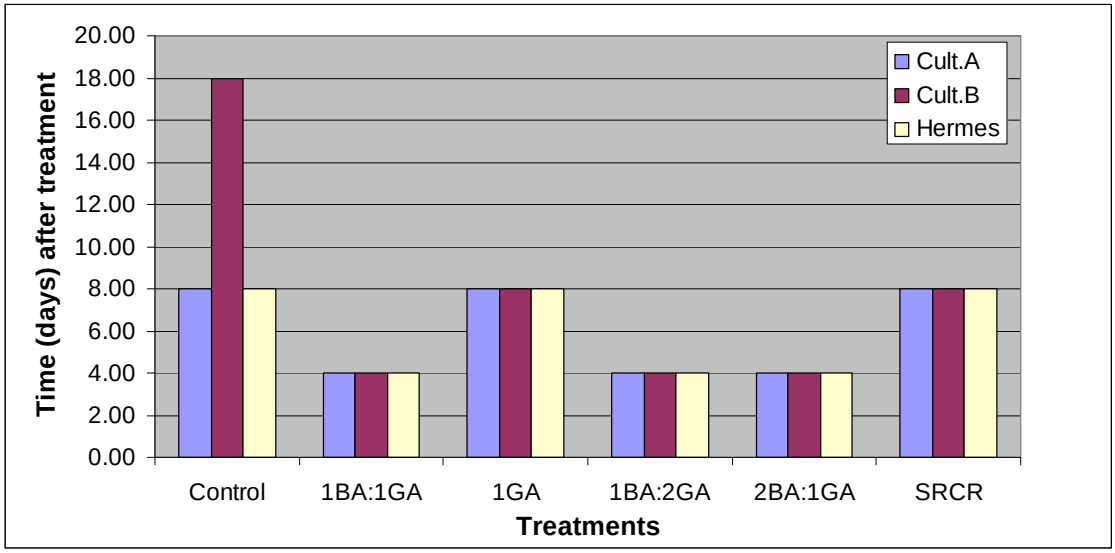

Figure 3.2: Time to 80% dormancy termination in tuber segments of Cult. A, Cult. B and Hermes after treatment with cytokinin and gibberellin

Data not statistically analyzed

The intact tubers sprouted considerably later than was the case with the tuber segments (Figure 3.3). In all three cultivars, untreated tubers took much longer to start sprouting and the application of gibberellin alone did not have an effect on dormancy.

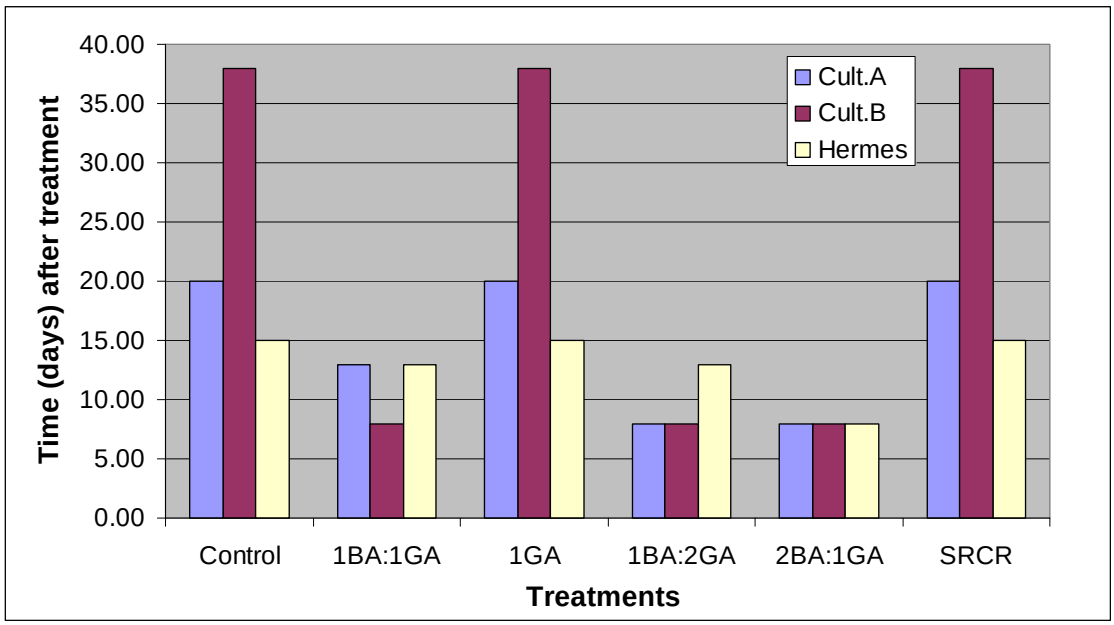

Figure 3.3: Time to 80% dormancy termination of intact tubers of Cult. A, Cult. B and Hermes after treatment with cytokinin and gibberellin

Data not statistically analyzed

In both Trial 1 and Trial 2 a combination of cytokinin and gibberellin terminated dormancy earlier than using only gibberellin, but there is a tendency for tubers to terminate dormancy earlier when cut and placed on a moist medium. In cultivars with a longer dormant period (Caren and Cult. B), gibberellin treatment tended to terminate dormancy earlier than the control, but in cultivars with a short dormant phase, gibberellins did not have a significant effect. It may be that gibberellin did not terminate dormancy, but only stimulated sprout growth after the physiological dormant phase had been terminated by endogenous factors. This may well be the case in Figure 3.2 which shows that cultivars with a lesser dormant phase did not react to gibberellin applications, whereas gibberellins may have stimulated the dormant bud to start sprouting as in Cult. B. Turnbull & Hanke (1985) reported that gibberellin treatments did not have an effect on dormancy termination but have a "functional role in growth of potato tuber buds". According to Suttle (2004b) gibberellins were found to be more active at the beginning of sprouting and were not responsible for termination of dormancy, but rather for the stimulation of/and further growth of sprouts.

In the trials GA_{4+7} was used to test for dormancy termination. Claassens & Vreugdenhil (2000) mentioned that GA_{4+7} can be used for sprout stimulation and growth. They did however, also mention that higher concentrations are needed in more dormant tubers to initiate sprouting. In our trials the treated tubers did not react significantly differently to higher or lower concentrations of gibberellin with regard to dormancy termination or the stimulation of sprouting.

The reaction of tubers to the application of cytokinin was variable, but dormancy was terminated within four to five days of treatment. Subsequent sprout growth was, however, much less than the tubers treated with gibberellins. Suttle & Banowetz (2000) also reported that cytokinin treatments resulted in dormancy termination within four days of application, but they injected ethanol with cytokinins into the tubers. In a recent article by Vreugdenhil *et al.* (2006) it was reported that ethanol had the same reaction as gibberellins in stimulating sprouting. Although Suttle & Banowetz (2000) and our own preliminary trials indicated that the control, a 5% ethanol solution, did not have an effect on dormancy termination, possible effects of ethanol cannot be ruled out. A combination of cytokinin and gibberellin resulted in more growth than cytokinin alone. Chen (1975) hypothesized that cytokinins may be responsible for the termination of dormancy by inhibiting the action of abscisic acid and thus allowing gibberellins to stimulate sprout growth. This may be the case in our trials which showed that dormancy was terminated by cytokinin, and that gibberellin stimulated sprout growth. Kucera, Cohn & Leubner-Metzger (2005) demonstrated that cytokinins, together with abscisic acid, played an important role in the germination of sorghum seeds, while gibberellin levels were very low. Cytokinin may have an important synergistic effect with other hormones in germination and the termination of dormancy. It appears that cytokinin may be the hormone responsible for the termination of dormancy, allowing other hormones to then stimulate further growth.

Trial 3

The cultivar Up-to-Date was used to establish whether tubers exhibit a time dependent reaction to sprout stimulation treatments. Intact tubers stored in a dark growth chamber at

20˚C did not sprout within the five weeks of the trial. In treated tubers, it was evident that sprouting commenced earlier when a combination of cytokinin and gibberellin was applied, although the time between treatment and sprouting was very short. At the first treatment date a combination of gibberellin and cytokinin terminated dormancy within three days and the time to terminate dormancy increased somewhat over the following two weeks before decreasing again by week 4. Dormancy was terminated relatively early throughout the trial regardless of the treatment applied, indicating that the specific batch of fresh greenhouse tubers may not have been in an endo-dormant state.

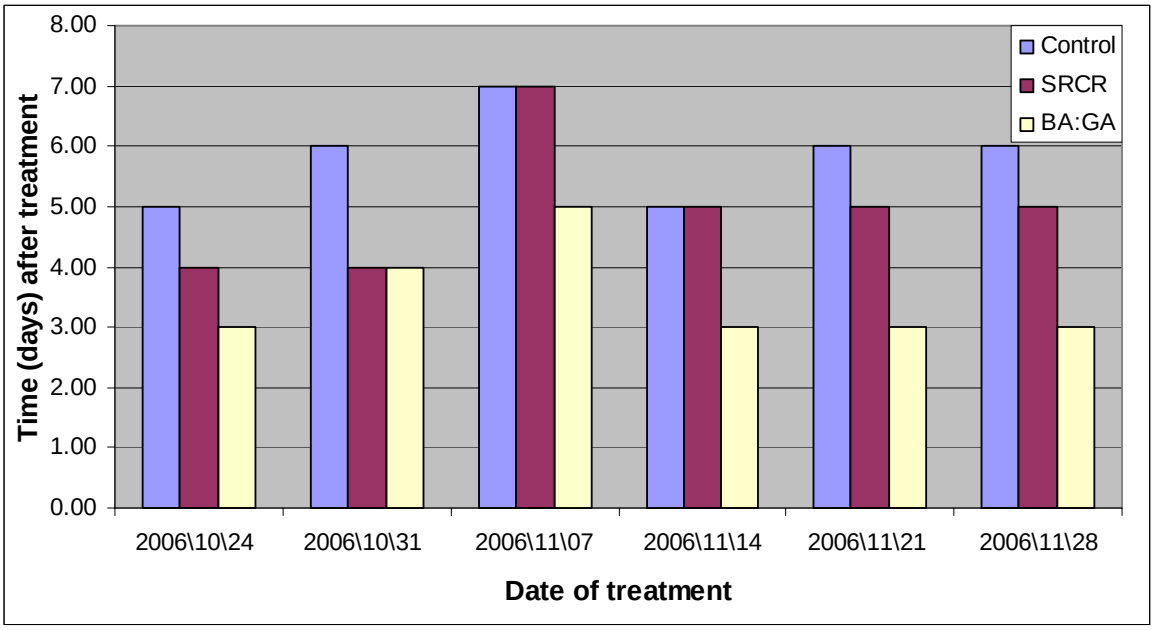

Figure 3.4: Time dependent termination of dormancy of cv. Up-to-date following application of gibberellin or a combination of cytokinin and gibberellin

Data not statistically analyzed

Coleman (1987) reported that cytokinin sensitivity is prevalent at the beginning and end of dormancy and tubers will be less sensitive to a cytokinin application between these two periods. According to Crabbé & Barnola (1996) cytokinins were more likely to break dormancy of dormant sweet cherry buds in the early phase of dormancy and gibberellins were only able to terminate dormancy near the end of the dormant phase.

In all three trials tubers were treated directly after harvest and dormancy was terminated within five days. It is of interest to note that Suttle (2001) reported that cytokinins did not have an effect on dormant tubers early in the dormant phase, but later the tubers reacted on a dose-dependent application. Koda (1982) reported that cytokinin levels decreased after harvest and only increased again shortly before the end of the dormant phase. Suttle (2004b) found that gibberellin concentration was high during the early period of dormancy and decreased during storage only to rise again during initiation of sprout growth.

3.5 CONCLUSIONS

Cytokinin, or a combination of cytokinin and gibberellin, terminated dormancy as soon as four days after treatment, provided tuber segments were placed on a moist medium. Cytokinin seems to be an important regulatory hormone in the termination of dormant buds. Gibberellin stimulates the growth of sprouts, inter alia by stimulating the synthesis of DNA and RNA. The concentrations of cytokinin and gibberellin to which the tubers were exposed in the trials had a small effect on their efficacy in terminating dormancy. Different ratios of cytokinin to gibberellin in the combined treatments did not affect their efficacy. It seems that cytokinins are responsible mainly for the termination of dormancy, by re-initiating the cell cycle and gibberellins are then responsible for further growth of the sprouts. In addition, cytokinins may create a sink region to attract assimilates from other parts of the plant/organ for the growing cells.

CHAPTER 4

EFFECT OF CYTOKININ AND GIBBERELLIN ON SPROUT GROWTH

4.1 ABSTRACT

The effect of cytokinin and gibberellin as well as cutting and tuber age on sprout growth of tubers of cv. Caren and Up-to-date was determined. Treatments consisted of either cytokinin (BAP) or gibberellin (GA_{4+7}) or a combination of the two. Tubers were either cut at the stolon end and the apical portions placed on moist cotton wool (Trial 1, 3 and 4) or tubers were kept dry and either cut at the stolon end and the apical portions used, or left intact (Trial 2). Tubers treated with a combination of cytokinin and gibberellin, or high concentrations of gibberellin (0.1 gL^{-1}), attained more sprout growth than tubers treated with only cytokinin (0.1 gL^{-1}) or a low concentration of gibberellin (0.005 gL^{-1}) (Trial 1 and 2). In Trial 2, tuber-segments produced longer sprouts than intact tubers, but the number of sprouts were less, while in Trial 3 tuber age did not affect the ability of tubers to sprout, but more sprout growth occurred when tubers were treated with cytokinin and gibberellin directly after harvest. A combination of cytokinin and gibberellin or high concentrations of gibberellin enhanced sprouting more than when only cytokinin was applied. Cutting tubers and supplying moisture enhanced sprout initiation and subsequent growth, whereas age of tubers did not affect the efficacy of sprout development.

4.2 INTRODUCTION

Plant growth regulators (PGR's) play an important role in the growth processes of plants. In particular, cytokinins and gibberellins are involved in initiation and maintenance of growth. Cytokinins are growth hormones responsible for cell division as well as differentiation (Mok & Mok, 2001; Hartmann *et al*, 2002) whereas gibberellins are responsible for cell elongation (Low, 1975; Gardner *et al,* 1985). Research has mainly focused on the effect of a particular growth regulator and seldom on the effect that a combination of growth regulators may have on sprout growth.

Most of the recent literature focuses on the genetic and biochemical effect of PGR's on the cellular functioning of plants. Cytokinins are responsible for the maintenance of the cell cycle, stimulating the G1-phase to proceed to the S-phase, and the transition from the G2 to the M-phase. These transitions occur through the synthesis of D-type cyclins that must bind to kinase proteins (Suttle, 1996; Francis & Sorrell, 2001; Mok & Mok, 2001; Horvath *et* al, 2003; Suttle, 2004a). Cytokinins are reported to be responsible for creating sink regions, attracting assimilates for growth processes (Sattelmacher & Marschner, 1978).

Gibberellins function in a regulatory role in controlling the levels of Cdc2 kinase (Francis & Sorrell, 2001). Also, gibberellins are involved in the synthesis of reducing sugars (Claassens & Vreugdenhil, 2000) which serves as an energy source for growing cells. Francis and Sorrell (2001) suggested that by inhibiting the flow of reducing sugars, the G1-to-S and G2-to-M transitions are inhibited and no cell growth will occur.

It seems that both cytokinin and gibberellin function in regulating the cell cycle at the transition points. Though a lot of research has gone into establishing the role of gibberellins and cytokinins, little information exist on the synergistic effect of these two PGR's. Low (1975) reported that when kinetin and GA_3 were applied in combination on lateral buds of *Phaseolus vulgaris* L. it increased the growth significantly compared to using either cytokinin or gibberellin separately. Kefeli (1978) also suggested that cytokinins function better when applied in combination with other growth hormones. Cytokinins interact with

other growth regulators in dormancy and germination regulation according to Kucera *et al.* (2005).

The objective of this chapter is to evaluate the effect of cytokinin and gibberellin, particularly in combination, on sprout growth of potato tubers. The effect of cutting of the tubers and tuber age on sprout growth of tubers treated with cytokinin and gibberellin is also evaluated.

4.3 MATERIALS AND METHODS

Tubers of cultivars Caren and Up-to-date were multiplied in greenhouses at the Experimental Farm of the University of Pretoria from March to September 2006. When tubers reached a size of approximately 20g the haulms were removed ten days before harvest to allow skin set. Harvested tubers were kept in a dark growth chamber at 20˚C and used within two weeks. Tubers were dipped in a 0.6gL^{-1} dimethyl didecyl ammonium chloride solution (Sporekill) to contain soft rot during experimentation, and left to dry. Tubers were then immersed in the treatment solution for one hour. The treatments consisted of either gibberellin (GA_{4+7}) or cytokinin (BAP) or a combination of the two. Tubers were checked daily for signs of sprouting. A tuber was considered sprouted when a sprout reached a length of 2mm. Intact tubers of cv. Caren remained dormant for at least eight weeks and cv. Up-to-date for at least six weeks. Four trials were conducted.

Trial 1: Effect of cytokinin and gibberellin on sprout growth

Twenty tubers of cv. Up-to-date and thirty tubers of cv. Caren per treatment were exposed to the following treatments:

• Control	Distilled water
• SRCR (Standard registered commercial rate)	0.005 gL^{-1} gibberellin
• 0.5BA	0.05gL^{-1} cytokinin
• 0.5BA:0.5GA	0.05 gL^{-1} cytokinin + 0.05 gL^{-1} gibberellin
• 0.5BA:1GA	0.05 gL^{-1} cytokinin + 0.1 gL^{-1} gibberellin
• 0.5GA	0.05 gL^{-1} gibberellin
• 1BA:0.5GA	0.1 gL^{-1} cytokinin + 0.05gL^{-1} gibberellin
• 1BA	0.1 gL^{-1} cytokinin
• 1BA:1GA	0.1 gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
• 1BA:2GA	0.1 gL^{-1} cytokinin + 0.2 gL^{-1} gibberellin
• 1GA	0.1 gL^{-1} gibberellin
• 2BA:1GA	0.2 gL^{-1} cytokinin + 0.1 gL^{-1} gibberellin

Tubers were cut at the stolon end and the apical portions placed on moist cotton wool in plastic containers in a dark growth chamber at 20˚C. Readings of cv. Up-to-date were taken 14 days after treatment. Cultivar Caren had two readings taken 20 and 32 days after treatment upon which sprouts were harvested to determine dry mass.

Trial 2: Effect of cytokinin and gibberellin on tubers not supplied with water

Ten tubers of cv. Caren were used for each treatment. Tubers were left intact or cut at the stolon end and the apical portion was then used. All the tubers were kept dry. Readings were taken 82 days after treatment. The treatments consisted of the following:

- Control — Distilled water
- SRCR — 0.005 gL^{-1} gibberellin
- 0.5BA — 0.05 gL^{-1} cytokinin
- 0.5BA:0.5GA — 0.05 gL^{-1} cytokinin + 0.05gL^{-1} gibberellin
- 0.5BA:1GA — 0.05 gL^{-1} cytokinin + 0.1 gL^{-1} gibberellin
- 0.5GA — 0.05 gL^{-1} gibberellin
- 1BA:0.5GA — 0.1 gL^{-1} cytokinin + 0.05 gL^{-1} gibberellin
- 1BA — 0.1 gL^{-1} cytokinin
- 1BA:1GA — 0.1 gL^{-1} cytokinin + 0.1gL^{-1} gibberellin
- 1BA:2GA — 0.1 gL^{-1} cytokinin + 0.2 gL^{-1} gibberellin
- 1GA — 0.1 gL^{-1} gibberellin
- 2BA:1GA — 0.2 gL^{-1} cytokinin + 0.1 gL^{-1} gibberellin

Trial 3: Effect of cytokinin and gibberellin concentration

Ten tubers of cv. Caren were used for each treatment. Readings were taken after 32 days. Treatments consisted of the following:

- Control — Distilled water
- BA:GA(2) — 0.002 gL^{-1} cytokinin + 0.002 gL^{-1} gibberellin
- BA:GA(4) — 0.004 gL^{-1} cytokinin + 0.004gL^{-1} gibberellin
- BA:GA(10) — 0.01 gL^{-1} cytokinin + 0.01gL^{-1} gibberellin
- BA:GA(20) — 0.02 gL^{-1} cytokinin + 0.02gL^{-1} gibberellin
- BA:GA(40) — 0.04 gL^{-1} cytokinin + 0.04gL^{-1} gibberellin
- BA:GA(60) — 0.06 gL^{-1} cytokinin + 0.06gL^{-1} gibberellin
- BA:GA(80) — 0.08 gL^{-1} cytokinin + 0.08gL^{-1} gibberellin

Trial 4: Effect of tuber age

Up-to-date tubers were harvested ten days after haulm removal and the first treatment were applied the next day (Time 1). Over the following five weeks (Time 2-6) ten tubers were treated every seven days. Readings were taken after seven days and treatments consisted of the following:

- Control — Distilled water
- SRCR — 0.005 gL^{-1} gibberellin
- BA:GA — 0.005 gL^{-1} cytokinin + 0.005gL^{-1} gibberellin

In all the trials treatments were completely randomized, and statistically analyzed using the SAS® program. The number of sprouts was taken as a co-variable to determine if the number of sprouts may have an influence on mean length per sprout and mean dry mass per tuber and to adjust the number of sprouts to a uniform number namely, the mean number of sprouts over the entire experiment. In this, the number of sprouts is accounted for. The data were analyzed using linear models. The explanatory variables in these models were:

- Trial 1: Hormonal treatments
- Trial 2: Hormonal treatments and cutting

- Trial 3: Hormonal treatments
- Trial 4: Hormonal treatments and time

The SAS PROC GLM procedure was used for the analysis. Significance of predictions was determined if P-values were smaller than 0.05. Estimated means and their standard errors were calculated using PROC GLM. Significance of differences amongst means was determined by using Fisher's protected procedure.

Data not presented in the Results section is summarized in Tables A 4.1-4.3 of the Appendix.

SAS®: SAS version 8.2 running under VM/CMI on the University of Pretoria mainframe computer

4.4 RESULTS AND DISCUSSION

Trial 1: Effect of cytokinin and gibberellin on sprout growth

The effect of the treatments on sprout growth of cv. Up-to-date is summarized in Table 4.1, while Figure 4.1 illustrates sprouting reaction. Both the high gibberellin treatments (0.5GA and 1GA) and combination treatments resulted in more sprouts per tuber and longer sprouts than the control or cytokinin treated tubers. Tubers treated with only cytokinin produced less sprouts per tuber and shorter sprouts than the gibberellin and cytokinin plus gibberellin treatments. Tubers treated with the high concentration of cytokinin (1BA) did not differ from the control in sprout length.

Table 4.1: Effect of cytokinin and gibberellin treatments on sprout number and length and dry mass of sprouts of Up-to-date tubers 14 days after treatment

Treatment (n = 20)	Mean sprout number/tuber	Mean length per sprout (mm)	Total sprout length per tuber	Mean sprout dry mass/tuber(g)	
				Sprout dry mass/tuber	With sprout number as co-variable
Intact tuber	0	0	0	0	0
Control	2.29 a	6.47 a	14.82	0.008 a	0.028 ab
SRCR	4.25 b	12.94 b	54.99	0.024 bc	0.034 bcd
0.5BA	7.92 d	14.64 b	115.95	0.032 cd	0.034 bc
0.5BA:0.5GA	8.73 de	20.36 c	177.74	0.036 de	0.035 bcd
0.5BA:1GA	10.35 ef	20.99 cd	217.25	0.042 def	0.034 bcd
0.5GA	9.64 def	24.38 de	235.02	0.051 fg	0.047 ef
1BA:0.5GA	9.33 def	24.30 de	226.72	0.043 efg	0.039 cde
1BA	5.51 c	8.38 a	46.17	0.015 ab	0.025 a
1BA:1GA	10.36 ef	23.10 cde	239.32	0.048 fg	0.041 cdef
1BA:2GA	11.02 f	24.04 cde	264.92	0.053 g	0.043 def
1GA	9.71 def	26.23 e	254.69	0.053 g	0.049 f
2BA:1GA	9.53 def	24.96 e	237.87	0.047 efg	0.043 cdef
SEM	0.07	1.4		0.004	0.003

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different (P<0.05), n=20.

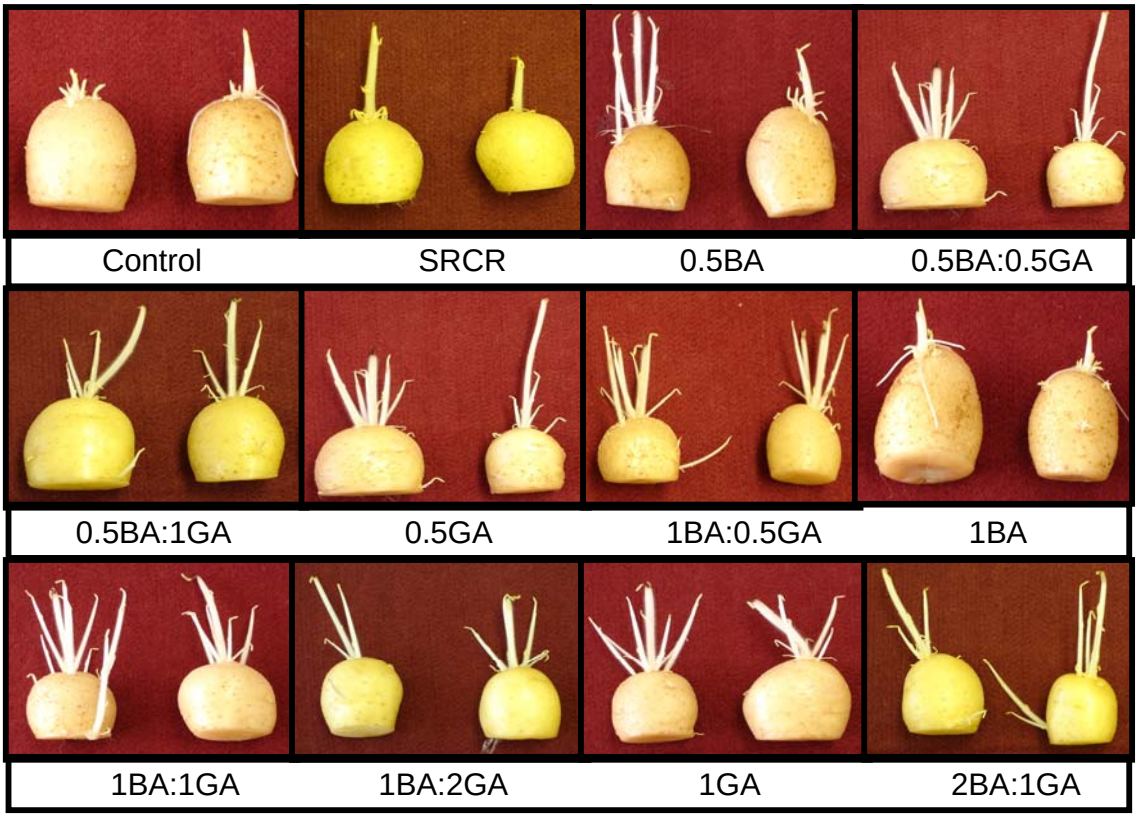

Figure 4.1: Effect of cytokinin and gibberellin treatment on sprout growth of Up-to-date tubers 14 days after treatment

The results indicate that a lower concentration of cytokinin (the 0.5BA treatment) resulted in more sprout growth (14.6 mm) than a higher concentration (1BA; 8.4 mm). Sprout lengths were reflected in the sprout dry mass data. Taking the number of sprouts as a co-variable of sprout dry mass still resulted in the same trend, with combinations of cytokinin and gibberellin (1BA:1GA, 1BA:2GA, 2BA:1GA) not differing from high concentrations of gibberellin, but with a higher dry mass than the control and cytokinin treatment 1BA.

The effect of the treatments on cv. Caren is summarized in Table 4.2, while Figure 4.2 illustrates the sprouting reaction to the treatments and in Table 4.3 the effect on sprout number and sprout dry mass is summarized. Tubers treated with either a combination of cytokinin and gibberellin or high concentrations of gibberellin had similar growth after 20 days, but with more sprout growth than the control, SRCR and cytokinin treated tubers. The only combination treatment that did not differ from the control, SRCR or cytokinin

treatments was 0.5BA:0.5GA. If the number of sprouts were taken as a co-variable, the same pattern was obtained, indicating that the number of sprouts did not have an influence on the mean length attained by the sprouts.

Table 4.2: Effect of cytokinin and gibberellin treatment on sprout length of cv. Caren measured at 20 and 32 days after treatment

Treatment (n = 30)	20 Days after treatment		32 Days after treatment	
	Mean length per sprout (mm)	With sprout number as co-variable	Mean length per sprout (mm)	With sprout number as co-variable
Intact tuber	0	0	0	0
Control	*4.33 ± 6.7 abc	2.26 ± 6.7 ab	5.73 ± 4.4 a	-0.85 ± 4.3 a
SRCR	6.97 ± 4.2 ab	5.47 ± 4.3 ab	21.71 ± 3.6 bcd	19.71 ± 3.5 cd
0.5BA	5.94 ± 3.1 a	6.15 ± 3.1 a	11.83 ± 3.6 ab	10.62 ± 3.4 bc
0.5BA:1GA	19.71 ± 3.2 d	20.15 ± 3.2 c	30.32 ± 3.6 def	33.29 ± 3.4 ef
0.5BA:0.5GA	15.77 ± 3.4 bcd	15.20 ± 3.3 bc	26.64 ± 3.7 cde	26.40 ± 3.6 de
0.5GA	22.71 ± 3.3 d	22.66 ± 3.3 c	35.95 ± 3.7 ef	36.97 ± 3.5 f
1BA:0.5GA	18.33 ± 3.2 cd	19.70 ± 3.2 c	19.81 ± 3.6 bc	23.03 ± 3.5 d
1BA	6.72 ± 3.7 ab	6.54 ± 3.7 ab	7.73 ± 4.1 a	7.18 ± 3.9 ab
1BA:1GA	20.89 ± 3.2 d	20.68 ± 3.1 c	36.42 ± 3.9 ef	35.04 ± 3.7 ef
1BA:2GA	21.11 ± 3.6 d	20.44 ± 3.6 c	23.26 ± 3.8 cd	21.01 ± 3.6 d
1GA	23.41 ± 3.3 d	22.53 ± 3.3 c	39.00 ± 3.6 f	39.56 ± 3.4 f
2BA:1GA	21.66 ± 3.1 d	23.59 ± 3.2 c	28.98± 3.7 cdef	32.88 ± 3.6 ef

* Mean ± standard error
Means within the same column sharing the same letters are not significantly different (P<0.05).

After 32 days tubers treated with a low concentration of gibberellin (SRCR, 21.7 mm) did not differ from the combination treatments, but had longer sprouts than the control (5.7 mm) and 1BA (7.7 mm) treated tubers. The co-variance analysis did not affect the pattern, indicating that the number of sprouts did not influence the mean length attained by the sprouts. The standard error of control treatments were higher and showed more variance than hormonal treatments, indicating that sprout growth was less uniform in control treatments.

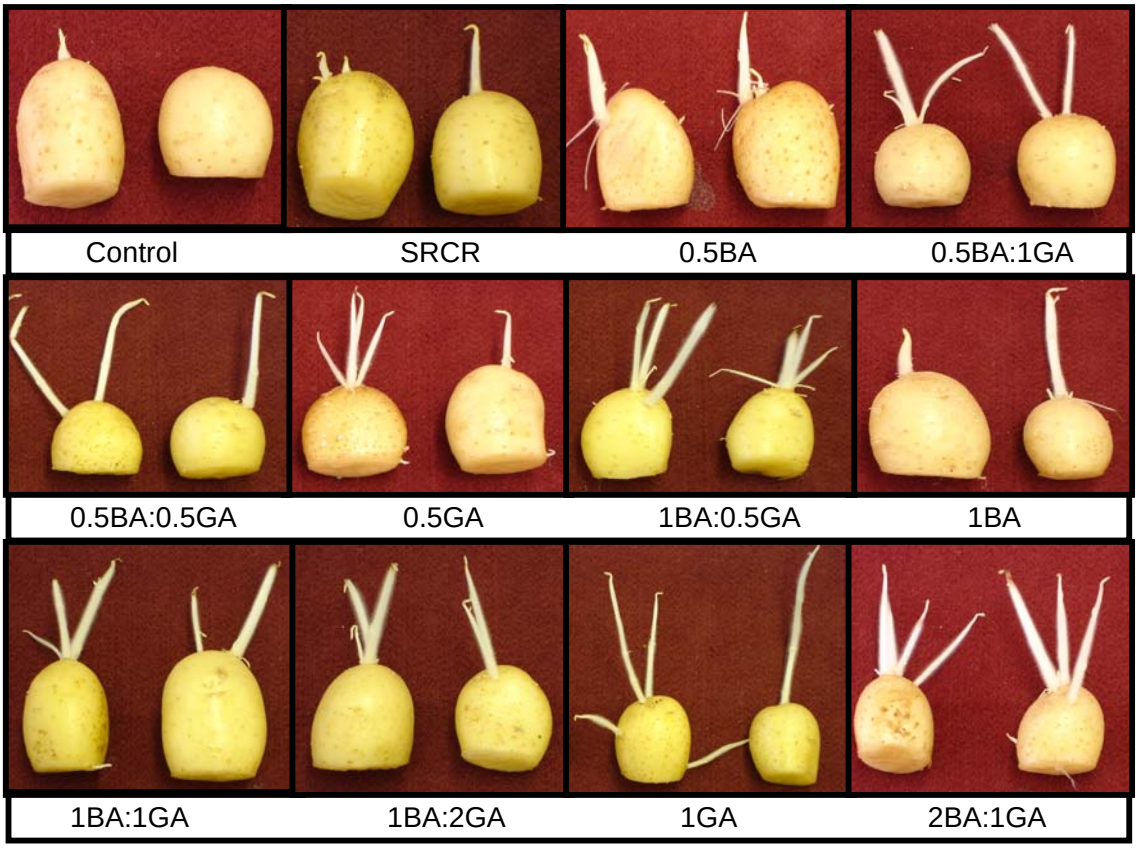

Figure 4.2: Effect of cytokinin and gibberellin treatment on sprout growth of Caren tubers 20 days after treatment

The mean number of sprouts and mean sprout dry mass per tuber of cv. Caren is summarized in Table 4.3. The control (0.21) and SRCR (0.71) treatments sprouted irregularly compared to the other treatments that typically had more than two sprouts per tuber after 20 days. Sprout numbers generally increased after 32 days, with the most prominent increase in the SRCR treatment (2.27). High concentrations of gibberellin (0.5GA; 1GA) resulted in more sprouts than similar concentrations of cytokinin (0.5BA; 1BA). The control, SRCR and 1BA treatments resulted in the lowest sprout mass per tuber, while gibberellin and combination treatments resulted in a significantly higher dry mass with the 2BA:1GA treatment producing the highest dry mass. Taking the number of sprouts as a co-variable did not have a prominent effect on the sprout dry mass results.

Table 4.3: Effect of cytokinin and gibberellin treatment on sprout number and dry mass of cv. Caren measured at 20 and 32 days after treatment

Treatment (n = 30)	Mean sprout number/tuber		Mean sprout dry mass/ tuber (g)	
	20 Days	32 Days	Mean sprout dry mass/ tuber	With sprout number as co-variable
Intact tuber	0	0	0	0
Control	0.21 a	0.72 a	0.003 a	0.010 a
SRCR	0.71 b	2.27 bc	0.015 abc	0.016 abc
0.5BA	2.96 fgh	2.49 bc	0.017 bcd	0.017 abc
0.5BA:1GA	2.69 efgh	3.72 d	0.027 de	0.023 bcd
0.5BA:0.5GA	1.89 cdef	2.44 bc	0.022 cde	0.022 bcd
0.5GA	2.26 cdefg	2.91 cd	0.027 de	0.026 cde
1BA:0.5GA	3.28 gh	3.73 d	0.024 cde	0.020 abcd
1BA	1.54 cd	1.85 b	0.008 ab	0.010 a
1BA:1GA	2.47 defgh	1.90 b	0.029 ef	0.031 de
1BA:2GA	1.41 c	1.84 b	0.011 ab	0.014 ab
1GA	1.80 cde	2.92 cd	0.027 cde	0.026 bcde
2BA:1GA	3.66 h	3.09 cd	0.040 f	0.037 e
SEM	0.12	0.10	0.004	0.004

SEM: Standard error of the mean
Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Dormancy must first be terminated before growth can occur. Dormancy can only be terminated by initiation of the cell cycle. Growth is a combination of cell division and enlargement. Cytokinins are mainly responsible for cell division and gibberellins for cell enlargement. During dormancy cells are mainly arrested in the G1 phase of the cell cycle. Unblocking of this phase can only commence if D-type cyclins are present. Cytokinins have been found to play an important role in synthesis of D-type cyclins (Francis & Sorrell, 2001). Thus it seems that cytokinins are mainly responsible for termination of dormancy and it has been shown in Chapter 3 that cytokinin treatments terminated dormancy earlier than using gibberellin.

Results indicated that cytokinin treated tubers had significantly less sprout growth than tubers treated with similar concentrations of gibberellin or a combination of the two. Although cytokinin terminated dormancy earlier than gibberellin treatments, subsequent sprout growth was slower. Suttle & Banowetz (2000) reported that cis-zeatin was responsible for dormancy termination, but cytokinin did not have an effect on further sprout growth. According to Beemster, Fiorani & Inzé (2003) cell division is a consequence, and not the cause, of growth. Traas & Bohn-Courseau (2005) are of the opinion that cell division will not necessarily result in growth. They mentioned that extra nuclei were not enough for extra cell volume and that cell division may only be necessary to replicate genetic material.

Applying gibberellin at the same concentration as cytokinin resulted in significantly more growth, but when gibberellins were supplied at very low concentrations (SRCR) little growth occurred. Gibberellins did not necessarily terminate dormancy but stimulated sprout growth after dormancy was reduced (Chapter 3). Francis & Sorrell (2001) hypothesized that gibberellin may be responsible for cell division by regulation of the G2-M phase of the cell cycle by altering the levels of Cdc 2 kinase. Gibberellins are also able to express B-type cyclins that are important in regulation of the G2-M phase (Doonan, 1996; Horvath *et al*, 2003). By supplying gibberellins to cells blocked in the G2-M phase, cell division and growth can commence.

Though gibberellin treatments did result in significantly more sprout growth, a combination of cytokinin and gibberellin resulted in similar sprout growth, and initiation of this growth was earlier (Chapter 3). A combination of cytokinin and gibberellin resulted in significantly more growth than using only cytokinin, or gibberellin at low concentration.

Suttle (2004a) mentioned that the endogenous gibberellin concentration only increased after dormancy had been terminated and sprouting commenced. Gibberellin may thus be responsible for further growth. Gibberellin functions in the synthesis of reducing sugars, by conversion of starch to glucose and fructose (Claassens & Vreugdenhil, 2000). During termination of dormancy a transition takes place whereupon a tuber becomes a source organ. During this transition starch is synthesized to sugars to be used by growing sprouts.

Cytokinins have been found to create sink regions to attract assimilates for sprout growth. According to Quiroz-Figueroa & Vázquez-Ramos (2006) a combination of sucrose and cytokinin are involved in D-type cyclin expression. Gutierrez *et al.* (2002) also linked D-type cyclins to nutrient availability. Cyclin D4 has been found to respond if sugars are available (Stals & Inzé, 2001).

According to Doonan (1996) the number of cells is not important for growth. Fewer cells are capable of maintaining growth, though more cells may increase the growth rate. This could well be the case when applying a combination of cytokinin and gibberellin. Cytokinins will terminate dormancy of dormant buds while attracting assimilates. Gibberellin may be involved in the mobilization of assimilates for growth as well as in the functioning of the cell cycle. When only cytokinins are applied to tubers little sprout growth occurs if the organ is still dormant and gibberellin levels are low. Low levels of gibberellin may limit the availability of assimilates for growth. The application of cytokinin will initiate dormant buds to start growing, but a further application of gibberellin will initiate the synthesis of reducing sugars to maintain growth of the initiated sprouts. Kefeli (1978) mentioned that there is an interaction between cytokinins and gibberellins and cytokinins require the addition of other hormones to function properly. Hill (1980) hypothesized that cytokinins are able to act upon a bud causing a change in growth and making it possible for gibberellin to act upon this bud increasing the growth rate. It seems that applying a combination of cytokinin and gibberellin may well be an answer to terminate dormancy earlier and increase growth.

Trial 2: Effect of cytokinin and gibberellin on tubers not supplied with water

Sprouting of the tubers in dry conditions commenced much later than when tuber-segments were placed on moist cotton wool (Trial 1). If tuber-segments and intact tubers are compared, the mean sprout length over all the treatments was 39 mm for tuber-segments compared to the 27 mm for intact tubers, with a P-value of 0.0006. Table 4.4 and Figure 4.3 clearly illustrate that the control, SRCR and cytokinin treatments did not differ, but exhibited shorter sprouts (generally <20mm) than those exposed to high concentrations of gibberellin as well as cytokinin: gibberellin combination treatments (typically 30-60 mm). In the case of intact

tubers, the analysis with the sprout number as co-variable illustrates that there were no differences between cytokinin, control and SRCR treatments, but with sprout lengths of all other treatments being significantly longer.

Table 4.4: Effect of cutting on sprout length of cytokinin and gibberellin treated Caren tubers 82 days after treatment

Treatment (n = 10)	Tuber-segments		Intact tubers	
	Mean length per sprout (mm)	With sprout number as co-variable	Mean length per sprout (mm)	With sprout number as co-variable
Control	8.97 a	-1.58 a	7.98 a	-6.37 a
SRCR	28.99 ab	23.39 b	16.51 ab	10.14 a
0.5BA	9.13 a	-4.38 a	17.10 ab	-1.25 a
0.5BA:0.5GA	52.64 bcde	49.03 cd	40.59 cd	43.53 b
0.5BA:1GA	68.25 de	61.66 cde	20.78 abc	35.03 b
0.5GA	59.41 cde	62.74 de	37.64 bcd	40.59 b
1BA:0.5GA	36.62 bc	49.85 cd	44.64 d	37.60 b
1BA	7.78 a	0.20 a	6.02 a	-3.67 a
1BA:2GA	46.99 bcd	53.29 cd	29.84 bcd	35.44 b
1GA	39.7 bc	43.02 bc	36.63 bcd	46.23 b
1BA:1GA	49.23 bcde	60.48 cde	31.01 bcd	39.94 b
2BA:1GA	66.6 de	78.85 e	42.86 d	37.15 b
SEM	9.8	7.1	7.7	6.1

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Table 4.5 summarizes the results for number of sprouts per tuber as well as the sprout dry mass per tuber. The trend for the control, SRCR and cytokinin to produce fewer sprouts than the gibberellin and combination treatments was similar to that of Trial 1. On average, tuber-segments produced 2.1 sprouts per tuber and intact tubers 3.2 ($P<0.0001$). The mean sprout dry mass per tuber showed the same trend as that of Table 4.4 with generally no significant differences between cytokinin, control and SRCR treatments, but sprout mass of all the other treatments were significantly more.

Table 4.5: Effect of cutting on sprout number and dry mass of cytokinin and gibberellin treated Caren tubers 82 days after treatment

Treatment (n = 10)	Sprout number/tuber		Sprout dry mass/ tuber (g)	
	Cut	Intact	Cut	Intact
Control	1.35 ab	1.45 ab	0.010 ab	0.009 a
SRCR	1.68 abc	2.36 bc	0.017 bc	0.012 ab
0.5BA	1.14 a	1.00 a	0.008 a	0.014 abc
0.5BA:0.5GA	1.96 abcd	3.45 cde	0.019 c	0.026 d
0.5BA:1GA	1.65 abc	5.23 e	0.019 c	0.025 d
0.5GA	2.39 bcd	3.13 cde	0.024 c	0.022 cd
1BA:0.5GA	3.35 d	2.34 bc	0.022 c	0.020 bcd
1BA	1.48 ab	1.99 abc	0.006 a	0.005 a
1BA:2GA	2.62 bcd	4.19 de	0.019 c	0.022 cd
1GA	2.48 bcd	4.37 de	0.024 c	0.024 d
1BA:1GA	2.95 cd	4.55 e	0.021 c	0.021 bcd
2BA:1GA	2.58 bcd	2.60 bcd	0.021 c	0.018 bcd
SEM	0.17	0.17	0.003	0.003

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Cutting generally increased sprout length of treated tubers (Table 4.4), but did not affect dry mass (Table 4.5). Intact tubers had more sprouts than tuber segments (Table 4.5), but the length of sprouts were shorter (Table 4.4). Reaction to hormonal treatments regardless of whether tubers were cut was similar to the reaction of tubers in Trial 1. Regardless of cutting, sprouting increased if a combination of cytokinin and gibberellin was applied. Cytokinin treatments resulted in less sprout growth. This trend is clearly seen in Table 4.4 (Intact B) where cytokinin treated tubers did not differ from the control or SRCR, but had significantly less growth than the other treatments. The question is why wounded tubers produced more sprout growth than intact tubers.

Mader, Emery & Turnbull (2003) observed that there was an increase in cytokinin concentration at the cut end of chickpea (*Cicer arietinum*) stems and the cytokinins identified

were able to promote cell cycling. They also observed that mitosis was initiated within an hour after cutting. In potato tubers cutting also has an effect on cytokinin synthesis. Koda (1982) indicated that wounding (in this case peeling) tubers increased cytokinin levels, an increase of 23 fold occurred after 24 hours. Thus it seems that cutting or wounding of tubers will increase cytokinin levels, and dormancy will be terminated earlier through initiation of the cell cycle.

Gibberellins have also been reported to increase after tubers have been cut. Clegg & Rappaport (1970) indicated that gibberellin concentration increased twelve hours after tubers had been wounded. They hypothesized that an increase in gibberellin concentration resulted in elevated levels of reducing sugars. An increase in gibberellin concentration has been associated with an increase in DNA synthesis (Shih & Rappaport, 1970), which could lead to increased sprout growth. According to Claassens & Vreugdenhil (2000) wounding increased sprouting and the hypothesis was that gibberellin might have been synthesized by wounding. It seems that wounding can increase both cytokinin and gibberellin concentrations, and the elevated levels of these two PGR's may lead to termination of dormancy and an increase in sprout growth. According to Slomnicki & Rylski (1964) cutting terminated dormancy earlier and the application of gibberellin together with cutting enhanced sprout growth.

Although tuber segments did show more sprout growth, tubers that were not supplied with water took much longer to sprout. In Trial 2 measurable sprout growth only occurred 60 days after treatment whereas the first readings of Trial 1 were taken 20 days after treatment. By supplying water, the rate of sprout initiation was increased. According to Kefeli (1978) PGR's are able to attract water and nutrients. It is hypothesized that cytokinins may create a sink region that would promote the transport of assimilates. Also, cytokinins are said to play a role in cell enlargement by the uptake of water through the decrease of osmotic potential caused by synthesis of reducing sugars (Arteca, 1996). Thus it seems that by cutting and/or applying additional cytokinin and gibberellin, synthesis of reducing sugars can commence and cell cycling can be initiated, but water must be supplied.

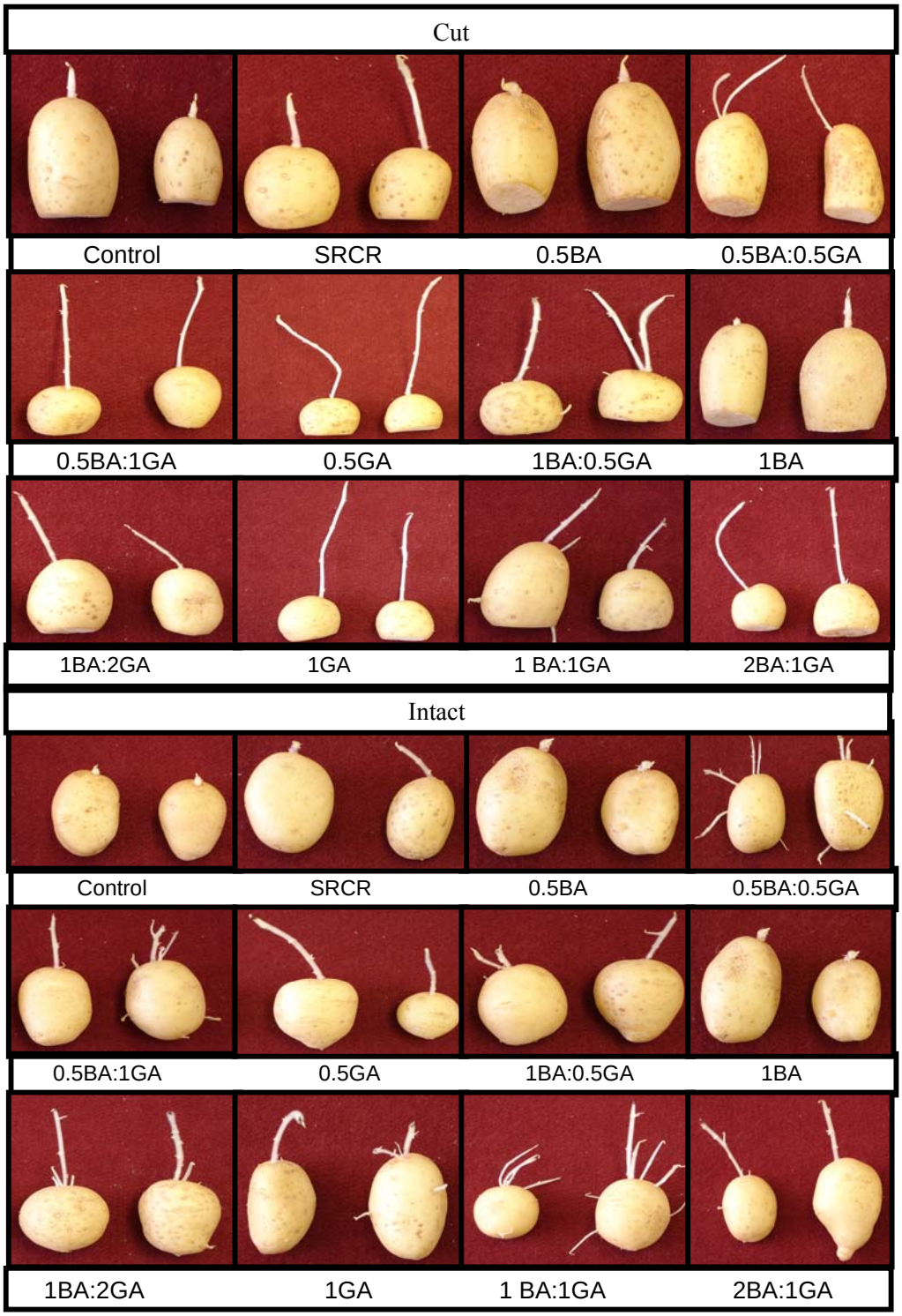

Figure 4.3: Effect of cutting on sprout growth of cytokinin and gibberellin treated Caren tubers 82 days after treatment

Trial 3: Effect of cytokinin and gibberellin concentration

Exposing tubers to cytokinin and gibberellin concentrations of $0.02gL^{-1}$ (treatment BA:GA(20)) or less did not improve sprouting compared to the control (Table 4.6 and Figure 4.4). Tubers exposed to concentrations lower than $0.02gL^{-1}$ cytokinin and gibberellin terminated dormancy 5 days later than tubers exposed to higher concentrations (BA:GA(60)). Treatment BA:GA(60) resulted in more sprouts, longer sprouts and a larger dry mass than practically all other treatments. Exposing tubers to higher concentrations (BA:GA(80)) did not improve sprouting when compared to treatment BA:GA(60).

Table 4.6: Effect of cytokinin and gibberellin concentration on sprout number, length, dry mass and time to 80% dormancy termination of Caren tubers

Treatment (n = 10)	Sprout number/tuber	Mean length per sprout (mm)	Sprout dry Mass/tuber (g)	Days to visual sprouting
Control	1.05 abc	11.81 ± 5.3 ab	0.005 ab	13
BA:GA(2)	1.00 ab	4.25 ± 5.0 a	0.002 ab	13
BA:GA(4)	0.71 a	7.82 ± 6.3 ab	0.001 a	13
BA:GA(10)	0.76 a	11.45 ± 5.7 ab	0.007 ab	13
BA:GA(20)	1.62 abc	19.22 ± 4.5 b	0.012 bc	10
BA:GA(40)	1.89 bc	11.28 ± 4.5 ab	0.008 ab	6
BA:GA(60)	2.16 c	32.11 ± 4.5 c	0.021 c	4
BA:GA(80)	1.87 bc	16.05 ± 5.0 ab	0.012 bc	4
SEM	0.18	5.83	0.004	

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

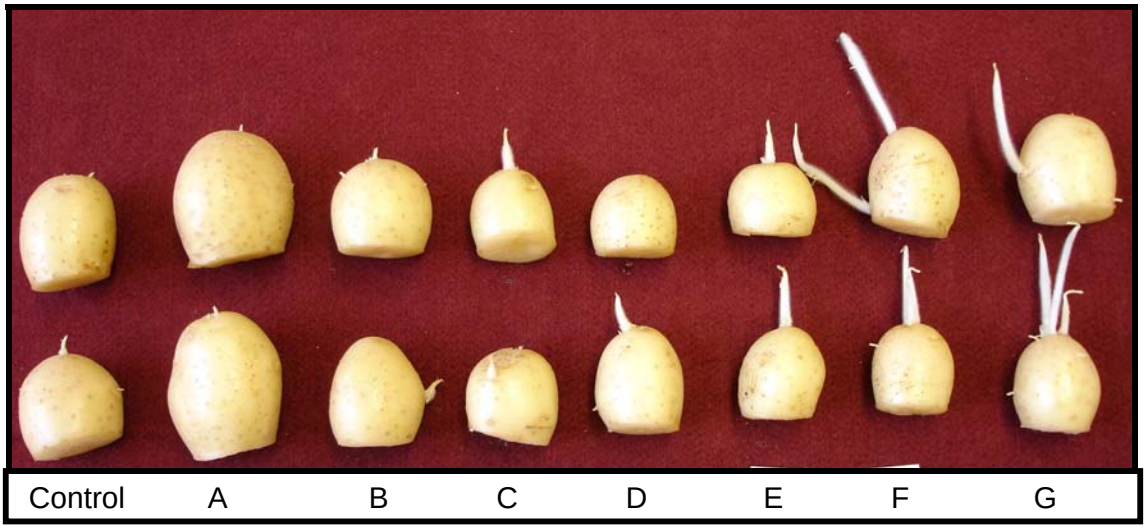

Figure 4.4: Effect of cytokinin and gibberellin concentration on sprout growth

It seems that the concentration of cytokinin needs to be relatively high to stimulate D-cyclins to initiate cell cycling and also to create strong sinks for assimilate transport to growing sprouts. The highest concentration led to less sprout growth as well as weak and malformed sprouts.

Trial 4: Effect of tuber age

Tubers treated a day after harvest developed longer sprouts than tubers treated in the five weeks thereafter (Figure 4.5 and Table 4.7). The control treatment did not show significant differences in sprout length over the five week trial period. Treatment SRCR had significantly longer sprouts at Time 1 (45.8 mm) than in the following weeks (mean 19.3 mm). There was a tendency for sprout length to increase at the end of the trial period. The same was observed for treatment BA:GA. The number of sprouts per tuber showed a similar trend with no differences occurring in the control treatment, but SRCR and BA:GA differing from week to week.

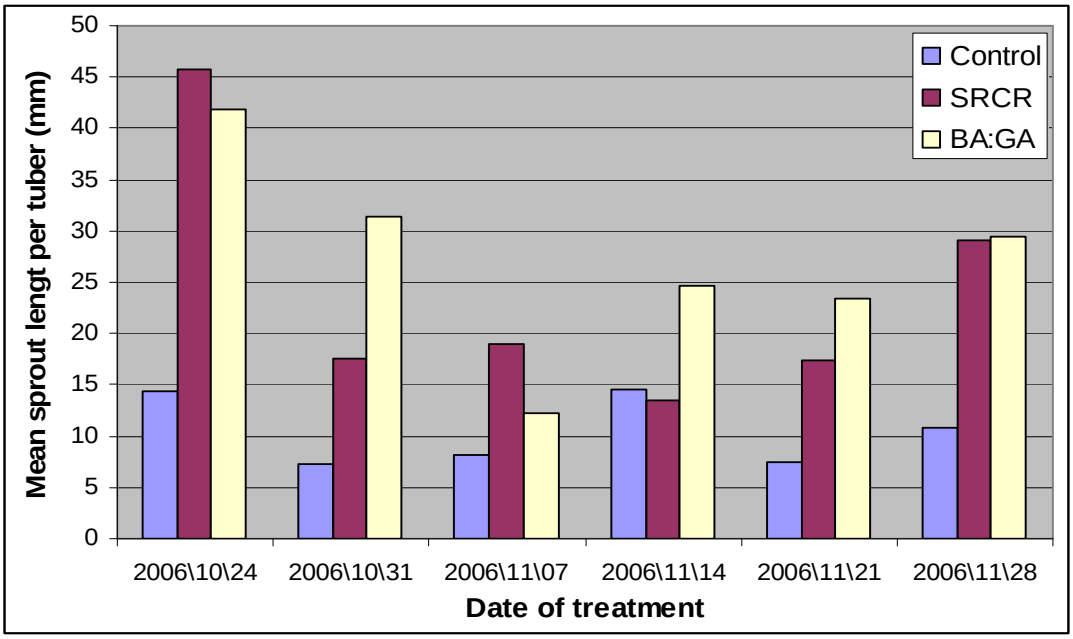

Figure 4.5: Effect of tuber age on sprout length of cytokinin and gibberellin treated Up-to-date tubers

Table 4.7: Effect of tuber age on sprout number and length of cytokinin and gibberellin treated Up-to-date tubers

	Mean Sprout Number/Tuber			Mean Sprout Length/Tuber (mm)		
	Treatment (n = 10)			Treatment (n = 10)		
Time	Control	SRCR	BA:GA	Control	SRCR	BA:GA
1	1.53 a	3.31 bc	3.02 bc	14.3 a	45.8 c	41.8 c
2	1.17 a	1.23 a	3.2 bc	7.3 a	17.5 ab	31.3 bc
3	1.14 a	3.54 bc	1.6 a	8.1 a	19 ab	12.2 a
4	1.23 a	2.05 ab	2.39 abc	14.5 a	13.5 a	24.7 b
5	1.14 a	1.64 a	1.96 ab	7.4 a	17.3 ab	23.4 ab
6	1.23 a	4.12 c	3.65 c	10.9 a	29.1 b	29.5 b
SEM	0.17	0.17	0.17	4.3	4.3	4.3

SEM: Standard error of the mean

Means within the same column sharing the same letter are not significantly differernt (P<0.05)

According to Suttle (2001) tubers react to a time-dependent increase in sensitivity to cytokinins and tubers did not react to cytokinin applications directly after harvest, but Coleman (1987) mentioned that tubers did react to cytokinins at the beginning and end of the dormant period.

Gibberellins have been found to stimulate sprouting only after dormancy has been terminated. According to Suttle (2004b) exogenously applied gibberellin will stimulate sprouting in a time- and dose dependent manner. From the results it is evident that freshly harvested tubers had more sprout growth, thus reacting better to application of cytokinin and gibberellin directly after harvest.

4.5 CONCLUSIONS

Both cytokinin and gibberellin may be involved in initiating sprout growth. Cytokinins are able to terminate dormancy by synthesis of D-cyclins, thus stimulating cell cycling. Cytokinins are also responsible for creating sink regions to attract assimilates for use by growing sprouts. Gibberellin functions in the latter part of the cell cycle, initiating the G2-M phase by synthesis of B-type cyclins. Gibberellins are important in the synthesis of reducing sugars, thus creating an energy source for growing sprouts.

By cutting or wounding tubers, cytokinins and gibberellins are reported to be synthesized. However, water is also needed to maintain an osmotic-potential gradient for the flow of hormones and assimilates.

CHAPTER 5

EFFECT OF CYTOKININ AND GIBBERELLIN ON SPROUT GROWTH RATE

5.1 ABSTRACT

The effect of cytokinin and gibberellin application, and regular removal of the wound periderm to facilitate water uptake by tuber segments, on subsequent sprout growth and sprout growth rate was evaluated. Tubers of cv. Up-to-date were either cut once at the stolon end or had the wound periderm removed every second day. Removing the wound periderm every second day resulted in less sprout growth than when tubers were only cut once. Hormonal treatments resulted in a higher growth rate 9 days after treatment, whereas control treatments reached a maximum growth rate after 19 days. After the maximum growth rate was attained a sharp decrease in growth rate occurred. The decreased growth rate may be attributed to closed plasmodesmata, although membrane permeability affecting availability of assimilates for further growth may also play a role.

5.2 INTRODUCTION

Dormancy in potato tubers are primarily regulated by the cell cycle, but plasmodesmata may be directly involved in initiating, maintaining and terminating dormancy. During dormancy initiation, plasmodesmata in bud meristems are blocked by callose (1,3-β-D-glucan) deposits (Zambryski & Crawford, 2000; Rinne *et* al, 2001; Horvath *et al*, 2003). Rinne *et al.* (2001) mentioned that cold conditions are necessary to restore cell-to-cell communication by initiating the synthesis of 1,3-β-D-glucanase and that gibberellins play a role in termination of dormancy when meristems are exposed to cold.

During tuber growth potato tubers act as sink organs, but when sprouting is initiated transition takes place and the tubers become a source of assimilates for the developing sprouts. Crabbé & Barnola (1996) reported that cells underlying the bud meristems act as strong sinks during dormancy, preventing entry of metabolites, and the meristems will stay dormant until the cells' sink strength diminishes. The reduction in sink strength will allow assimilate flow towards meristimatic cells and facilitate sprouting. During this transition to a source, the plasmodesmata become branched (Kobayashi *et al*, 2005). Zambryski & Crawford (2000) hypothesized that branched plasmodesmata may decrease the outward flow of assimilates, but according to Schulz (2005) branched plasmodesmata decreases backflow of assimilates, thus regulating flow towards sink cells.

Plant growth regulators play an important role in the cell cycle, but also in the regulation of cell-to-cell communication. Rinne *et al.* (2001) stated that gibberellins may have a role in the transcription of 1,3-β-D-glucanase, removing the callose deposits and reinitiating cell-to-cell communication. The functioning of cytokinins in regulation of cell-to-cell communication is not clearly known. Kefeli (1978) mentioned that translocation of cytokinins between plant tissues is weak, but cytokinins can create attraction sites to attract assimilates from adjacent cells. Cytokinins have also been found to play a role in the synthesis and increase of 1,3-β-D-glucanase (Barwe, Sathiyabama & Jayabaskaran, 2002) and may have a synergistic role with gibberellins. Further it is evident that calcium has a function in source-sink regulation

(Roitsch, 1999) and together with cytokinins (creating attraction sites for assimilates) may function in stimulating growth through creation of strong sink cells.

Quantification of sprout growth rates can supply valuable, though indirect, information regarding the probable role of plasmodesmata in controlling the cell-to-cell movement of metabolites, hormones and other growth substances. According to literature exogenously applied cytokinin and gibberellin may function in removing callose deposits from blocked plasmodesmata allowing assimilate flow towards the dormant meristem cells. Removal of the wound periderm of tuber segments may increase water uptake, as well as stimulate synthesis of endogenous gibberellins and cytokinins, thus improving sprout growth.

5.3 MATERIALS AND METHODS

Tubers of cv. Up-to-date were multiplied in greenhouses at the Experimental Farm of the University of Pretoria. When tubers reached a size of approximately 20g the haulms were removed ten days before harvest to allow skin set. Harvested tubers were kept in a dark growth chamber at 20˚C and used within two weeks. Tubers retained in the growth chamber sprouted after 50 days. Ten tubers per treatment were dipped in a 0.6 gL^{-1} dimethyl didecyl ammonium chloride solution (Sporekill) to contain soft rot during experimentation, left to dry and thereafter immersed in the treatment solutions for one hour. The treatments consisted of the following:

A: Wound periderm allowed to develop on tuber segments

- Control Distilled water
- 1BA:1GA 0.1$g.L^{-1}$ cytokinin + 0.1 $g.L^{-1}$ gibberellin
- 1BA:2GA 0.1 $g.L^{-1}$ cytokinin + 0.2 $g.L^{-1}$ gibberellin
- 2BA:1GA 0.2 $g.L^{-1}$ cytokinin + 0.1 $g.L^{-1}$ gibberellin

B: Wound periderm regularly removed

- Control Distilled water
- 1BA:1GA 0.1$g.L^{-1}$ cytokinin + 0.1 $g.L^{-1}$ gibberellin
- 1BA:2GA 0.1 $g.L^{-1}$ cytokinin + 0.2 $g.L^{-1}$ gibberellin
- 2BA:1GA 0.2 $g.L^{-1}$ cytokinin + 0.1 $g.L^{-1}$ gibberellin

The treated tubers were cut at the stolon end and the apical portion positioned with the wound surface on moist cotton wool. In Group B the tubers were treated similarly except the thin layer of wound periderm was removed every second day to facilitate the uptake of water. Tubers were placed in plastic containers in a dark growth chamber at 20˚C. Tubers were considered sprouted when sprouts reached a length of 2 mm. Sprouts were measured eleven times during 24 days.

Tubers were treated on 2006/09/27 and sprout lengths were measured at the following times:

- Time 1 2006/10/02
- Time 2 2006/10/03
- Time 3 2006/10/04
- Time 4 2006/10/06
- Time 5 2006/10/09
- Time 6 2006/10/11
- Time 7 2006/10/13
- Time 8 2006/10/15
- Time 9 2006/10/17
- Time 10 2006/10/20
- Time 11 2006/10/24

The trial was completely randomized and statistically analyzed. The analysis of variance was determined separately for each time interval, and for the combined data using PROC GLM. The data were analyzed using linear models. Significance of predictions was determined if P-values were smaller than 0.05. Significance of differences amongst means was determined by using Fisher's protected procedure.

A Gompertz curve was drawn using the equation $y(t) = Ae^{-Br^t}$ where:

y = Sprout length at time t

t = Time

A = Upper asymptote

B = X-axis placement parameter

r = Average growth rate

From this equation the maximum growth rate could be determined using the first differential

$y' = -ABLn_e r * r^t e^{-Br^t}$

The time at which maximum growth rate is attained could be derived from the second differential $y'' = (-ABLn_e r)\left\{r^t Ln_e r * e^{-Br^t} + r^t(-BLn_e r * r^t * e^{-Br^t})\right\}$ setting equal to 0 and then solving for t, where $t_{max..} = \frac{Log_{10}\left(\frac{1}{B}\right)}{Log_{10} r}$

The parameters A, B and r was determined for 200 bootstrap replications and standard errors for the estimated values could then be determined.

Data not presented in the Results section is summarized in Tables A 5.1-5.4 of the Appendix.

5.4 RESULTS AND DISCUSSION

The Gompertz curve (Figure 5.1) illustrates the growth patterns of the sprouts. Both the controls had less growth than tubers treated with combinations of cytokinin and gibberellin, but the growth was more linear and had a steady increase (up to 10mm at the end of the trial). The growth of tubers treated with cytokinin and gibberellin increased rapidly (generally between 25 mm and 33 mm after 16 days), but then showed a decrease and after 27 days sprout growth had almost come to a halt.

The maximum growth rate of the sprouts could be deducted from the Gompertz equation as well as the time at which the maximum growth rate was attained. Tubers that were allowed to develop wound periderm (A) reached higher growth rates than tubers where the wound periderm was removed on a regular basis (B), but the differences were not significant (Table 5.1). Figure 5.2 illustrates the tendency for sprouts of tubers treated with combinations of cytokinin and gibberellin to reach a higher growth rate than the controls. The only significant differences that occurred were between the controls (mean1.35 mm day^{-1}) and tubers treated with combinations of cytokinin and gibberellin (mean 7.4 mm day^{-1}). No significant differences occurred between combinations of cytokinin and gibberellin treatments.

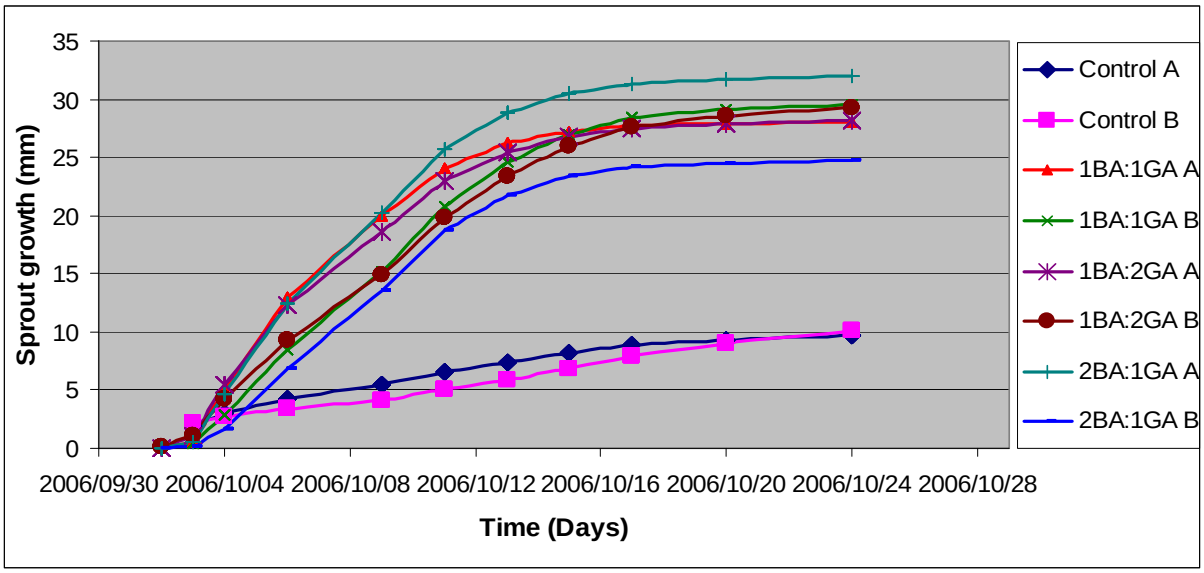

Figure 5.1: Gompertz growth curves of the effect of the treatment conditions on sprout length

Table 5.1: Comparison of time to maximum growth rate, maximum growth rate, length at maximum growth rate and upper asymptote of tubers treated with combinations of cytokinin and gibberellin in Group A and B

Treatments (n = 10)	Time intervals to maximum growth rate (days)		Maximum growth rate (mm/time unit)		Length at maximum growth rate (mm)		Upper asymptote (mm)	
Control A	*4.70 ±3.3 (11.1)	abc	1.34 ±0.36	a	4.93 ±4.13	abc	13.41 ±11.24	abc
Control B	12.13 ±8.9 (27)	abc	1.36 ±0.67	a	15.62 ±19.67	abc	42.47 ±53.47	abc
1BA:1GA A	3.72 ±0.14 (8.4)	a	8.58 ±2.3	b	10.49 ±0.61	abc	28.52 ±1.66	abc
1BA:1GA B	4.38 ±0.18 (10.1)	c	6.93 ±0.73	b	11.05 ±0.7	bc	30.04 ±1.9	bc
1BA:2GA A	3.75 ±0.12 (8.5)	a	7.50 ±1.73	b	10.48 ±0.5	b	28.48 ±1.35	b
1BA:2GA B	4.35 ±0.2 (10.05)	bc	5.89 ±1.13	b	11.15 ±0.54	bc	30.32 ±1.46	bc
2BA:1GA A	3.93 ±0.09 (8.9)	ab	8.58 ±1.03	b	11.87 ±0.42	c	32.26 ±1.13	c
2BA:1GA B	4.39 ±0.14 (10.2)	c	6.96 ±1.71	b	9.18 ±0.44	a	24.95 ±1.18	a

* Mean ± standard error
Means within the same column sharing the same letters are not significantly different ($P<0.05$).

The time it took to attain maximum growth was significantly less for tubers that were allowed to develop a wound periderm (mean 8.6 days) than similar treatments of tubers that had their wound periderm removed (mean 10.1 days). Because of the linear growth of the two controls, the length of sprouts at the time of maximum growth rate and the asymptote did not differ from tubers treated with combinations of cytokinin and gibberellin. Also, the time at which maximum growth rate of control treatments was attained did not differ significantly from cytokinin and gibberellin treatments.

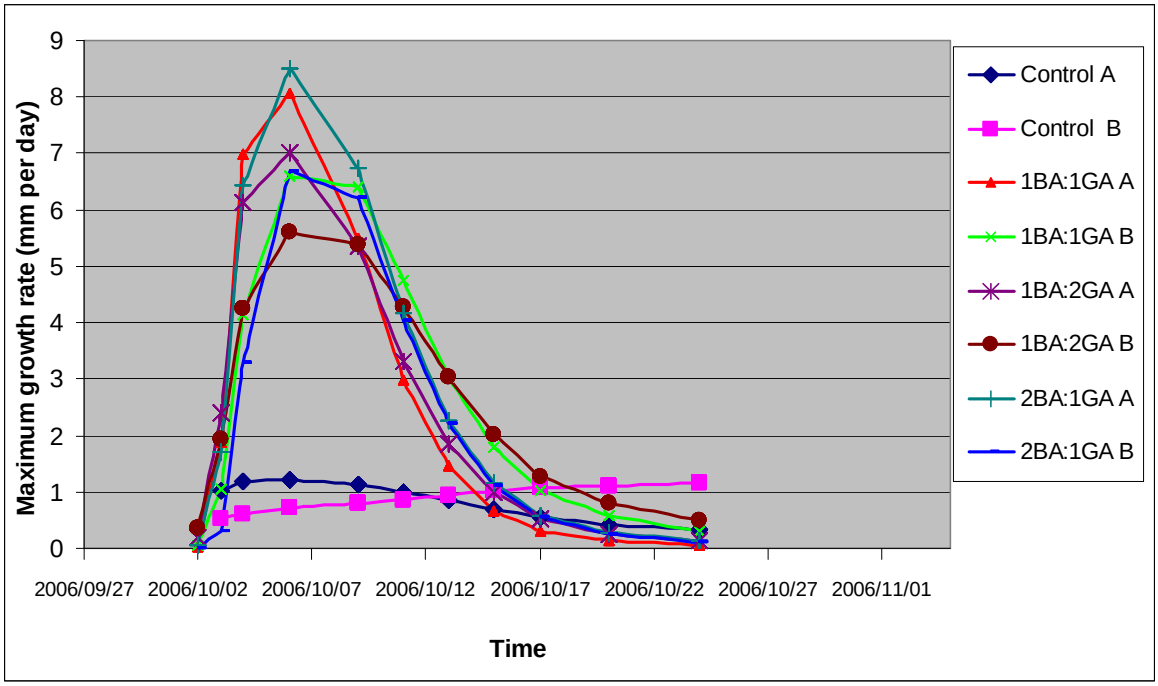

Figure 5.2: Sprout growth rate of tubers treated with combinations of cytokinin and gibberellin in Group A and B

Tubers that were allowed to develop wound periderm reached a maximum growth rate earlier than tubers that had their wound periderm frequently removed. According to Blackman & Overall (2001) plasmodesmata may be blocked by callose deposits during wounding. They mentioned that sieve plates of pea roots could be blocked by callose within a minute after wounding. It would seem that by frequently wounding tubers, the plasmodesmata may be closed for a longer duration and inhibit flow of assimilates. The frequent removal of the wound periderm resulted in shorter sprouts. If wounded, plasmodesmata of adjacent cells will close, provided there is no loss of turgor pressure in these cells. If turgor pressure is reduced in adjacent cells, leakage of cell sap into wounded cells will occur and communication between cells would continue (Epel, 1994). According to Schulz (2005) plasmodesmata would be closed if a large difference in turgor pressure existed between adjacent cells. Tubers that were allowed to develop a wound periderm may have opened their plasmodesmata after restoration of wounded cells and could acquire more water for transport of hormones and assimilates.

All the hormonal treatments had higher growth rates than control treatments. Tubers treated with combinations of cytokinin and gibberellin took an average of 9.4 days to reach a maximum growth rate, after which there was a reduction in growth, whereas the control treatments continued to have a more linear increase in growth. The increase in growth rate when treated with cytokinin and gibberellin could be a result of non-dormant cells. During the dormant phase there are still a small percentage of cells that are active (Coleman, 1987). Cells arrested in the G1-phase are mainly responsible for the dormant state, whereas cells in the G2-phase may be able to undergo mitosis. About 13% of cells are in the G2-phase of the cell cycle (Suttle, 1996). According to Doonan (1996) β-type cyclins may play an important role in regulation of the G2-phase. Gibberellins and certain cytokinins have been found to express β-type cyclins (Horvath *et al*, 2003). The number of cells may not be critical in growth, but if more cells were to be available, growth would be quicker (Doonan, 1996). Traas & Bohn-Courseau (2005) hypothesized that a possible function of the cell cycle is to supply DNA for continued growth. It was also shown that cell growth could occur without cell division. By only cutting tubers and supplying moisture to maintain the osmotic potential, growth may be initiated (as in the control treatments), but the application of cytokinin and gibberellin may increase the number of cells able to complete the cell cycle, thus increasing the growth rate.

Chapter 4 discussed the probability of cytokinin and gibberellin being synthesized when tubers were cut and these hormones may initiate growth of dormant buds. But, as seen in Figure 5.2, sprout growth of control treatments was significantly less than those of hormonal treated tubers. The application of cytokinin and gibberellin may initiate more cells to start cycling and as a result, increase sprout growth. Before cells can be initiated to start cycling, they must first be stimulated to go through the gap phases (G1 and G2). Cyclins are responsible for the transition of G1 to S and G2 to M- phase and can be stimulated by the application of cytokinins and gibberellins. D-type cyclins, which is important in G1 to S transition and is stimulated by cytokinins, is also important in the sensing of sugars (Traas & Bohn-Courseau, 2005). Before cytokinins can act upon cells, initiating the cell cycle by the synthesis of D-type cyclins, they must first reach the cells.

Cell-to-cell communication and transport of sugars and hormones take place through plasmodesmata. During dormancy, cell communication is blocked by callose deposits at the entry points of plasmodesmata. No flow of sugars and hormones can commence until the plasmodesmata are open. Unblocking of plasmodesmata may commence through the application of gibberellin. Rinne *et al.* (2001) suggested that gibberellin is involved in the control of dormancy when a plant is exposed to cold conditions. Though modern day potato cultivars do not need cold to terminate dormancy, the same mechanism applies. During cold exposure, internal gibberellin levels increase. Gibberellin has been associated with the synthesis of 1,3-β-D-glucanase, an enzyme that is responsible for the breakdown of callose (1,3-β-D-glucan)(Rinne *et al*, 2001; Rinne & Van der Schoot, 2004). The external application of gibberellin may stimulate the synthesis of 1,3-β-D-glucanase and unblock plasmodesmata. Rinne *et al.* (2001) did however show that gibberellin injected into dormant apical meristems did not move through blocked plasmodesmata within 6 hours, but they hypothesized that it may take longer for unblocking to occur. This may well be why the first signs of growth were only detected after 5 days.

If gibberellins are able to unblock plasmodesmata, transport of cytokinins and D-type cyclins to dormant cells can occur. These cells will then be initiated to complete the cell cycle. Besides unblocking of plasmodesmata, gibberellins will also be necessary in synthesis of reducing sugars that would be transported through plasmodesmata to growing cells. Cytokinin has also been found to promote the formation of plasmodesmata and thus directly influencing the communication between cells (Ormenese *et al*, 2006).

It seems that cytokinin and gibberellin application may unblock plasmodesmata and increase flow of assimilates to dormant cells. These cells would then be stimulated by cytokinin and gibberellin to complete the cell cycle and increase the number of cells that can maintain growth. Sprout growth was initiated as soon as 5 days after treatment with cytokinin and gibberellin, and a maximum growth rate was generally attained after 9.4 days. Growth was drastically reduced after the maximum growth rate was attained. The reason for this decline in growth rate may be described in terms of availability of assimilates.

Assimilates used for rapid sprout growth may have been attained from non-bound sources. The greater percentage of assimilates may still be bound and not readily available for transport through the plasmodesmata. An increase in non-bound or free assimilates is found when tubers start to sprout and is directly associated with membrane permeability (De Weerd, Hiller & Thornton, 1995). The tubers used in the trial were freshly harvested and dormant and thus had insignificant leakage of electrolytes.

If growth would commence as quickly as it did in the trials, a large gradient in assimilate concentration would develop from tuber towards the apical meristem. According to Schulz (2005) a large concentration gradient would result in a loss of sugars to surrounding cells. This gradient would result in the closure of plasmodesmata and the transport phloem would be isolated.

Calcium has been found to play an important role in plasmodesmal functioning. Elevated levels of calcium will lead to plasmodesmal closure (Zambryski & Crawford, 2000) and callose deposition may be a result of intercellular changes in calcium concentration (Roberts, 2005). According to Rinne & Van der Schoot (2004) changes in membrane potential could result in activation of calcium channels at the neck region of plasmodesmata. Increase in calcium concentration will lead to callose deposition. Sources of calcium can be found in the cell wall as well as the endoplasmic reticulum. Closure of plasmodesmata through the increase in calcium concentration could occur within seconds (Blackman & Overall, 2001). Saure (2005) reported that generally rapid growth is attained with high levels of gibberellin, and with a high growth rate a decrease in calcium is typically found. It is hypothesized that a high concentration gradient towards the growing meristem would lead to a high influx of calcium which would eventually lead to plasmodesmal closure.

According to Roberts (1988) soybean hypocotyls treated with cytokinin increased calcium transport towards treated parts. Cytokinins have also been found to enhance the ion influx of calcium (Barwe *et al*, 2002). The enhanced transport of calcium towards growing meristems caused by the application of only cytokinin may well be the cause of reduced growth

observed in Chapter 4 and may be the regulatory factor in decreasing the growth rate of tubers treated with cytokinin and gibberellin.

5.5 CONCLUSIONS

Frequency of wounding had an effect on subsequent sprout growth, but contrary to the original hypothesis removal of the wound periderm every second day resulted in a reduction in sprout growth compared to tubers that were only cut once. The reason for this reduced growth may well be in the closure of plasmodesmata. Plasmodesmata may be closed for a longer period and inhibit flow of assimilates and growth hormones.

Hormonal treatments resulted in high sprout growth rates possibly due to an increase in assimilate flow towards growing meristems. Gibberellins function in opening plasmodesmata by removal of callose and the synthesis of reducing sugars. Cytokinin creates a sink region that attract assimilates, and cytokinins may function in the formation of plasmodesmata, thus increasing the flow of assimilates towards the growing meristems.

The later decline in sprout growth may also result from the closure of plasmodesmata caused by a high turgor gradient. The high turgor gradient may have been caused by the high growth rate and plasmodesmata would close to restore the turgor pressure to its previous state. Depletion of freely available assimilates may have contributed to the declining growth rate.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSIONS

The potato industry represents an important division of South African agriculture. The annual potato crop represents approximately R2 billion (http://www.potatoes.co.za). Production costs of potatoes are relatively high (about R50 000/ ha under irrigation). Virus diseases can cause a large reduction in yield and can be introduced into a field by seed potatoes. The testing of seed potatoes for specific viruses is therefore of utmost importance. The seed certification scheme specifies tolerance levels for pests and diseases, and seed must be certified before it is dispersed to farmers. Seed tubers can only be tested for certain virus diseases once dormancy has been terminated and the tubers are sprouting. Tubers have a natural dormant period and will not sprout before dormancy is terminated.

Effect of cytokinin and gibberellin on tuber dormancy

A combination of cytokinin and gibberellin terminated dormancy earlier than only gibberellin (Chapter 3). The age of tubers (for the first 30 days) generally did not affect the efficacy of cytokinin and gibberellin to initiate sprouting. Tubers treated with only cytokinin sprouted earlier, but subsequent sprout growth was greatly reduced compared to combination treatments. According to Turnbull & Hanke (1985) gibberellin treatments did not have an effect on termination of dormancy, but had a 'functional role in growth of potato tuber buds'. It would seem that gibberellins are not responsible for termination of dormancy per se, but are more active in sprout initiation (Suttle, 2004b).

Cytokinin treated tubers initiated sprouting earlier than gibberellin treatments. Cytokinins have been found to be active in the regulation of D-type cyclins. D-type cyclins are responsible for the transition of the G1 to S-phase of the cell cycle. Dormant cells are generally arrested in the G1 phase of the cell cycle and by the application of cytokinin; D-type cyclins will initiate the cell to complete its cycle. Growth can then occur and dormancy will be terminated. According to Suttle & Banowetz (2000) cytokinins are responsible for termination of dormancy but have no effect on further sprout growth.

Effect of cytokinin and gibberellin on sprout growth

Although cytokinins are responsible for cell division and differentiation (Mok & Mok, 2001) by the synthesis of D-type cyclins (Suttle, 1996), they do not have an effect on further sprout growth (Suttle & Banowetz, 2000). Cytokinins are also responsible for the creation of sink regions (Sattelmacher & Marschner, 1978) that would attract assimilates to growing cells.

Gibberellins are responsible for cell elongation (Low, 1975) and synthesis of reducing sugars (Claassens & Vreugdenhil, 2000). Gibberellin treated tubers resulted in more sprout growth than cytokinin treated tubers, although buds took longer to start sprouting.

Combination treatments of cytokinin and gibberellin resulted in significantly more sprout growth than control or cytokinin treated tubers. The application of both cytokinin and gibberellin resulted in earlier sprouting, probably because cytokinins initiate cell cycling and stimulate flow of assimilates to growing meristems. Gibberellins function in synthesizing more sugars to maintain growth as well as functioning in cell elongation.

Effect of tuber wounding and availability of moisture on sprout growth

Tubers that were not cut had significantly less sprout growth than tubers that were cut. Cutting influences the endogenous concentrations of cytokinin and gibberellin and may have a direct effect on sprout initiation and growth. It has been found that wounding leads to an increase in cytokinin (Koda, 1982; Mader *et al*, 2003) as well as gibberellin (Clegg & Rappaport, 1970; Claassens & Vreugdenhil, 2000).

Although cutting of tubers resulted in more sprout growth, placing tubers on a moist medium had a much greater impact on sprout initiation and growth. Tubers that were left dry, took much longer to sprout. Cytokinins may play a role in cell enlargement by the uptake of water through the decrease of osmotic potential caused by the synthesis of reducing sugars by gibberellins (Arteca, 1996). Treating tubers with cytokinin and gibberellin, as well as cutting and supplying moisture, will thus result in significantly more sprout growth.

Influence of cytokinin and gibberellin on sprout growth rate

Treatments with cytokinin and gibberellin resulted in significantly higher growth rates than control treatments. The increase in growth rate can be ascribed to the increase in cells able to complete the cell cycle. An increase in cell number would lead to an increase in growth rate (Doonan, 1996). By applying cytokinin, D-type cyclins would be synthesized and will stimulate the G1 to S-phase transition. Cytokinins are able to attract assimilates through the creation of a sink region (Sattelmacher & Marschner, 1978). Creation of a sink region is accomplished by D-cyclins that senses reducing sugars (Traas & Bohn-Courseau, 2005). Cytokinins are also able to promote the formation of plasmodesmata (Ormenese *et al*, 2006). Although cytokinins can create sink regions, initiate cell cycling and promote formation of plasmodesmata, it is not yet clear if cytokinins can unblock plasmodesmata and initiate transport through it.

Gibberellins may be involved in the synthesis of 1,3-β-D-glucanase, an enzyme responsible for the breakdown of callose (1,3-β-D-glucan) (Rinne *et al*, 2001; Rinne & Van der Schoot, 2004). Gibberellins are also involved in the synthesis of reducing sugars (Claassens & Vreugdenhil, 2000). It is postulated that gibberellins unblock plasmodesmata and stimulate the synthesis of reducing sugars, allowing cytokinins to reach dormant cells and initiate the completion of the cell cycle and thus initiate growth, as well as attracting assimilates to maintain growth.

Frequently removing the wound periderm of a tuber segment leads to a reduced growth rate. The reduction in growth compared to tubers that were cut only once can possibly be ascribed to the blocking of plasmodesmata. Callose deposits accumulate at the neck region of plasmodesmata when plants are wounded and inhibit flow of assimilates through the plasmodesmata (Blackman & Overall, 2001).

After an initial increase in growth rate, a decrease occurred. The decrease in growth could be a result of plasmodesmal closure. Closure of plasmodesmata can be a result of a concentration gradient between the growing meristem and the underlying cells. Transport between tunica and corpus cells can only take place through the plasmodesmata (Rinne *et al*, 2001). According to Schulz (2005) a large difference in turgor pressure between adjacent cells would result in closure of plasmodesmata. Free assimilates could be depleted, causing a reduction in growth rate and influx of calcium may result in the closure of plasmodesmata.

CONCLUSIONS

A combination of cytokinin and gibberellin will terminate dormancy earlier (within 4-5 days) and will increase sprout growth, whereas gibberellin stimulated sprouting 9 days after treatment. The application of only cytokinin will terminate dormancy earlier than using only gibberellin, but subsequent sprout growth will be hampered.

Cutting influences dormancy termination and subsequent growth through the synthesis of cytokinin and gibberellin, but increasing the frequency of cutting may decrease the growth rate possibly because of callose deposits blocking flow of assimilates through plasmodesmata. Although cutting on its own have an increase in sprout growth, the availability of additional moisture will increase flow of assimilates towards growing meristems.

Although dormancy can be terminated within a relatively short time, subsequent sprout growth and development of a physiological normal plant is still hampered by various factors of which the plasmodesmata and hormones impacting on the cell cycle and plasmodesmata play a pivotal role. Currently researchers are focusing on these topics but no clear answers have yet been obtained as to the functioning mechanisms.

In conclusion, a combination of cytokinin and gibberellin will terminate dormancy earlier and increase growth. This will be of great importance in the potato seed industry, speeding up the process of virus testing and seed certification.

Aspects that need further investigation:

In the course of the study various aspects for future research opportunities have been identified of which the most important are outlined:

1. The effect of cytokinin and gibberellin treatments on reducing sugar content of tubers as well as the flow and availability at the growing meristems needs further investigation.

2. Gibberellins are able to unblock plasmodesmata (Rinne *et al*, 2001) but the role of cytokinins in relation to unblocking plasmodesmata is yet to be determined.

3. It has been shown that wounding initiates callose deposition and subsequent blocking of plasmodesmata (Blackman & Overall, 2001). But the functioning of plasmodesmata in tuber segment cells as well as the growing meristems of these tubers has to be determined.

4. Calcium plays an important role in cell functioning. Cytokinins have been found to increase calcium influx towards growing cells (Roberts, 1988). An increase in calcium would result in closure of plasmodesmata (Zambryski & Crawford, 2000). The influence of calcium in closure of plasmodesmata as well as the concentration in dormant and growing cells requires further investigation.

5. Although dormancy can now be terminated within a few days after treatment with a combination of cytokinin and gibberellin, it is still to be determined whether it would be possible to detect virus infections in such forced sprouts.

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APPENDIX

Table A 4.1: Effect of tuber age on sprout length, number and dry mass of cytokinin and gibberellin treated Up-to-date tubers

7 Days							
Mean Sprout Length /Tuber							
Treatments (n = 10)	1	2	3	4	5	6	SEM
Control	14.3 a	7.3 a	8.1 a	14.5 a	7.4 a	10.9 a	4.3
SRCR	45.8 b	17.5 a	19.0 a	13.5 a	17.3 ab	29.1 b	4.3
BA:GA	41.8 b	31.3 b	12.2 a	24.7 a	23.4 b	29.5 b	4.3
Mean Sprout Number/Tuber							
Treatments (n = 10)	1	2	3	4	5	6	SEM
Control	1.53 a	1.17 a	1.14 a	1.23 a	1.14 a	1.23 a	0.17
SRCR	3.31 b	1.23 a	3.54 b	2.05 a	1.64 a	4.12 b	0.17
BA:GA	3.02 b	3.20 b	1.60 a	2.39 a	1.96 a	3.65 b	0.17
14 Days							
Mean Sprout Length /Tuber							
Treatments (n = 10)	1	2	3	4	5	6	SEM
Control	14.4 a	20.7 a	35.2 a	32.5 a	13.8 a	48.5 a	11.6
SRCR	114.0 b	43.5 ab	78.0 b	60.3 a	51.3 b	47.8 a	11.6
BA:GA	87.8 b	59.3 b	45.8 ab	44.7 a	54.3 b	22.2 a	11.6
Mean Sprout Number/Tuber							
Treatments (n = 10)	1	2	3	4	5	6	SEM
Control	1.35 a	1.52 a	1.80 a	2.11 a	1.25 a	3.08 a	0.23
SRCR	4.14 b	1.46 a	5.02 b	3.32 a	2.63 ab	3.30 a	0.23
BA:GA	3.40 b	2.63 a	2.14 a	3.04 a	3.14 b	1.00 b	0.23
Mean Sprout Dry Mass/Tuber							
Treatments (n = 10)	1	2	3	4	5	6	SEM
Control	0.007 a	0.008 a	0.025 a	0.024 a	0.008 a	0.011 a	0.003
SRCR	0.028 b	0.02 b	0.025 a	0.026 a	0.019 b	0.021 b	0.003
BA:GA	0.025 b	0.026 b	0.026 a	0.022 a	0.028 b	0.02 ab	0.003

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Table A 4.2: Effect of tuber age on sprout number of cytokinin and gibberellin treated Up-to-date tubers

	Mean Sprout Number/Tuber					
	Treatment (n = 10)					
	7 Days			14 Days		
Time	Control	SRCR	BA:GA	Control	SRCR	BA:GA
1	1.53 a	3.31 bc	3.02 bc	1.35 ab	4.14 b	3.40 b
2	1.17 a	1.23 a	3.2 bc	1.52 ab	1.46 a	2.63 b
3	1.14 a	3.54 bc	1.6 a	1.80 ab	5.02 b	2.14 ab
4	1.23 a	2.05 ab	2.39 abc	2.11 ab	3.32 b	3.04 b
5	1.14 a	1.64 a	1.96 ab	1.25 a	2.63 ab	3.14 b
6	1.23 a	4.12 c	3.65 c	3.08 b	3.30 b	1.00 a
SEM	0.17	0.17	0.17	0.23	0.23	0.23

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Table A 4.3: Effect of tuber age on sprout length of cytokinin and gibberellin treated Up-to-date tubers

	Mean Sprout Length/Tuber					
	Treatment (n = 10)					
	7 Days			14 Days		
Time	Control	SRCR	BA:GA	Control	SRCR	BA:GA
1	14.3 a	45.8 c	41.8 c	14.4 a	114.0 c	87.8 c
2	7.3 a	17.5 ab	31.3 bc	20.7 ab	43.5 a	59.3 bc
3	8.1 a	19.0 ab	12.2 a	35.2 ab	78.0 b	45.8 ab
4	14.5 a	13.5 a	24.7 b	32.5 ab	60.3 ab	44.7 ab
5	7.4 a	17.3 ab	23.4 ab	13.8 a	51.3 ab	54.3 ab
6	10.9 a	29.1 b	29.5 b	48.5 b	47.8 ab	22.2 a
SEM	4.3	4.3	4.3	11.6	11.6	11.6

SEM: Standard error of the mean

Means within the same column sharing the same letters are not significantly different ($P<0.05$).

Table A 5.1: Comparison of mean sprout length per tuber between Group A and B within each time interval

Mean sprout length per tuber (mm)			
Time	A (n = 40)	B (n = 40)	P-value
1	2.00	2.00	---------
2	3.09 ± 0.15	2.41 ± 0.18	0.0062
3	5.01 ± 0.23	3.97 ± 0.29	0.0079
4	11.33 ± 0.54	7.11 ± 0.68	<0.0001
5	22.71 ± 1.09	16.60 ± 1.37	0.0013
6	24.54 ± 1.4	20.03 ± 1.42	0.0529
7	27.03 ± 1.12	22.47 ± 1.42	0.0164
8	27.40 ± 1.24	24.63 ± 1.57	0.1745
9	28.53 ± 1.20	25.93 ± 1.52	0.1872
10	29.21 ± 1.19	26.86 ± 1.51	0.2296
11	31.07 ± 0.90	27.59 ± 1.14	0.022

Differences with a P-value smaller than 0.05 is significantly different

Comparisons are made within rows

Table A 5.2: Effect of cytokinin and gibberellin treatments on average number of sprouts per tuber within each time interval

Treatments (n = 10)						
Time	Control	1BA:1GA	1BA:2GA	2BA:1GA	SEM	P-value
1	0.00 a	1.14 b	2.82 c	1.51 b	0.11	0.0022
2	0.59 a	2.67 b	4.09 b	3.56 b	0.15	0.0002
3	1.46 a	6.15 b	7.41 b	7.61 b	0.13	0.0195
4	3.48 a	8.24 b	9.13 b	9.48 b	0.10	0.9040
5	5.69 a	8.55 b	9.76 b	10.84 b	0.08	0.0248
6	6.06 a	8.24 b	7.92 ab	10.07 b	0.10	0.5834
7	5.65 a	8.04 bc	7.04 ab	10.11 c	0.01	0.8162
8	5.73 a	7.66 ab	6.38 a	9.29 b	0.11	0.2736
9	6.02 ab	7.74 bc	4.77 a	9.22 c	0.12	0.1508
10	5.35 ab	7.64 bc	4.76 a	9.25 c	0.13	0.0635
11	6.49 ab	8.54 bc	5.01 a	10.45 c	0.13	0.5458

SEM: Standard error of the mean

Means within the same row sharing the same letters are not significantly different ($P < 0.05$)

P-values smaller than 0.05 had significant differences within each time interval

Table A 5.3: Comparison between consecutive time intervals for means of mean sprout length and number per tuber using repeated measures (n = 80)

Mean sprout length per tuber (mm)				Mean number of sprouts per tuber		
Time	Mean	Comparisons	P-value	Mean	Comparisons	P-value
1	2.00	1 and2	<0.0001	1.13	1 and2	<0.0001
2	2.82	2 and 3	<0.0001	2.41	2 and 3	<0.0001
3	4.63	3 and 4	<0.0001	4.97	3 and 4	<0.0001
4	9.65	4 and 5	<0.0001	7.14	4 and 5	0.0004
5	20.23	5 and 6	0.0061	8.5	5 and 6	0.1312
6	22.82	6 and 7	0.0028	7.96	6 and 7	0.1450
7	25.25	7 and 8	0.0087	7.56	7 and 8	0.0017
8	26.38	8 and 9	0.0053	7.16	8 and 9	0.2301
9	27.54	9 and 10	0.0636	6.76	9 and 10	0.4273
10	28.33	10 and 11	0.0395	6.54	10 and 11	0.0042
11	29.76			7.37		

P-values smaller than 0.05 shows differences between means are significant

Table A 5.4: Comparisons between consecutive time intervals between Group A and B for mean number of sprouts per tuber using repeated measures

Mean number of sprouts per tuber (n = 40)					
Time	A	B	Difference	Comparisons	P-value
1	1.52	0.80	0.72	1 and2	0.0454
2	3.46	1.61	1.85	2 and 3	0.0102
3	5.89	4.18	1.71	3 and 4	0.0076
4	7.19	7.10	0.09	4 and 5	0.0237
5	7.70	9.4	-1.70	5 and 6	0.0939
6	7.70	8.20	-0.50	6 and 7	0.2230
7	7.70	7.50	0.20	7 and 8	0.0030
8	7.60	6.70	0.90	8 and 9	0.5460
9	7.40	6.14	1.26	9 and 10	0.3582
10	7.50	5.72	1.78	10 and 11	0.0274
11	7.70	7.10	0.60		

P-values smaller than 0.05 shows differences are significant