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RESPONSE OF POTATO TO PACLOBUTRAZOL AND MANIPULATION OF REPRODUCTIVE GROWTH UNDER TROPICAL CONDITIONS

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ABSTRACT

High temperature limit successful potato cultivation in the lowlands of tropical regions. One effect of high temperature may be an increase in gibberellin activity that is inhibitory to tuberization. Paclobutrazol blocks gibberellin biosynthesis and reduces its level in the plant. The effect of paclobutrazol on potato was examined under non-inductive conditions in a greenhouse and under field conditions in the hot tropical lowlands of eastern Ethiopia. Paclobutrazol was applied as a foliar spray or soil drench at rates equivalent to 0, 2, 3, and 4 kg a. i. per ha.

Paclobutrazol increased chlorophyll *a* and *b* content, and photosynthetic efficiency, enhanced early tuber initiation, delayed physiological maturity, and increased tuber fresh mass, dry matter content, specific gravity and crude protein content. It reduced the number of tubers per plant and extended the tuber dormancy period. Paclobutrazol reduced shoot growth, and plant height, and increased the partitioning of assimilates to the tubers while reducing assimilate supply to the leaves, stems, roots and stolons. Stomatal conductance and the rate of transpiration were reduced. In addition, paclobutrazol treatment increased tuber N, Ca and Fe content while reducing P, K and Mg content. Growth analyses indicated that paclobutrazol decreased leaf area index, crop growth rate, and total biomass production. It increased

specific leaf weight, tuber growth rate, net assimilation rate, and partitioning coefficient (harvest index). Microscopic observations showed that leaves of treated plants developed thicker epicuticular wax layers. The epidermal, palisade and spongy mesophyll cells were larger. It increased the thickness of the cortex and the size of vascular bundles and pith cells of the stem. It also increased the width of the cortex and favoured the formation of more secondary xylem vessels, resulting in thicker roots. Deposition of starch grains in the stem pith cells, and cortical cells of the stem and root, were stimulated in response to paclobutrazol treatment. In most instances the method of application did not affect the efficiency of paclobutrazol.

The effect of cultivar and reproductive growth on growth, photosynthetic efficiency, water relations, dry matter production, tuber yield and quality of potato was also the subject of investigation. Non-flowering, flowering and fruiting plants of cultivars Al-624, Al-436, CIP-388453-3(A) and CIP-388453-3(B) were evaluated under field conditions of a sub-humid tropical highland of eastern Ethiopia. Cultivars exhibited differences with respect to leaf stomatal conductance, rate of transpiration, net photosynthesis, biomass production and allocation, tuber yield, tuber size distribution, specific gravity, dry matter content and nutrient composition. Fruiting plants had higher leaf stomatal conductance, and higher rates of transpiration and photosynthesis rates. The leaf area index, tuber growth rate, and partitioning coefficient (harvest index) of the fruiting plants were reduced, but crop growth rates and net assimilation rates were higher. Without affecting total dry matter production, fruit development reduced the amount partitioned to the leaves, stems, roots, and tubers. Fruit development reduced total and marketable tuber mass and tuber numbers.

The effect of MCPA and paclobutrazol were studied under greenhouse and field conditions. Single foliar sprays were applied during the early and full bud development stages at rates of 0, 250, 500, and 750 g a.i. ha^{-1}. Both MCPA and paclobutrazol greatly reduced the number of flowers and completely inhibited berry set. MCPA did not affect the number, yield, dry matter content and specific gravity of tubers. Without affecting the number of tubers, paclobutrazol increased tuber yield, dry matter content and specific gravity.

Keywords: Anatomical modification, assimilate partitioning, Ethiopia, growth analysis, high temperature, non-inductive, paclobutrazol, potato genotypes, photosynthetic rate, *Solanum tuberosum* L, specific gravity, tropical lowland, tuber quality, tuber yield

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CHAPTER 1

GENERAL INTRODUCTION

The potato is one of mankind's most valuable food crops. In volume of production (347 million metric tons annually) it ranks fourth in the world after maize, rice and wheat, with an estimated production area of 18.9 million hectare (FAOSTAT data, 2004). Among root crops potato ranks first in volume produced and consumed, followed by cassava, sweet potato, and yam (FAOSTAT data, 2004).

The relatively high carbohydrate and low fat content of the potato makes it an excellent energy source for human consumption (Dean, 1994). The tuber is known to supply carbohydrate, high quality protein, and a substantial amount of essential vitamins, minerals, and trace elements (Horton & Sawyer, 1985). Moreover, the potato crop provides more nutritious food per unit land area, in less time, and often under more adverse conditions than other food crops. It is said to be one of the most efficient crops in converting natural resources, labour and capital into a high quality food with wide consumer acceptance (Horton, 1980).

The cultivated potato belongs to the family Solanaceae, genus *Solanum,* and section Tuberarium (Correll, 1962). The potato has its origin in the high Andes of South America and was first cultivated in the vicinity of Lake Titicaca near the present border of Peru and Bolivia (Horton, 1987). It was introduced to Ethiopia in 1858 by the German botanist Schimper (Pankhurst, 1964). Since then, the potato has become an important crop in many parts of the country.

Ethiopia, with an area of about 1.1 million square km and a total population of 67.7 million, is the fourth largest country in Africa, and is located within 3-15^{o}N latitude and 33-48^{o}E longitude. Agriculture is the mainstay of the economy and accounts for half of the gross domestic product, 85% of export earnings, and more than 80% of the total employment (http://www.nationmaster.com/country/et/Economy). The climate of Ethiopia is tropical monsoon with large topographic-induced variations. Based on temperature and moistures regimes the country has been classified into 18 major and 49 sub agro-ecological zones (Figure1.1). About 65% of the land area is situated in moist, sub-humid, humid and per-humid agro-ecologies. The remaining 35% is semi-arid with high temperatures throughout the year (EARO/ARTP, 1999). Although approximately two-thirds of the country is arable, only 15% of the area is presently under cultivation, and about 3% of the 3.5 million hectares of potentially irrigable land is being irrigated (http://www.madeinethiopia.net).

Ethiopia has suitable edaphic and climatic conditions for the production of high quality ware and seed potatoes. About 70% of the available agricultural land is located at an altitude of 1800-2500 m.a.s.l and receives an annual rainfall of more than 600 mm, which is suitable for potato production (Solomon, 1987). However, the current total area under potato production is estimated at 36, 736 ha with an annual production of 385, 258 metric tons. The national average yield is approximately 10.5 tons/ha, which is very low compared to the world average of 16.4 tons/ha (FAOSTAT data, 2004). A number of production problems that account for the small area cropped with potato and the low national yield have been identified. The major ones are the concentration of potato cultivation in the highlands with very little in the lowlands, lack of well-adapted cultivars, unavailability and high cost of seed tubers, non optimal agronomic practices, the prevalence of diseases and insect pests, and inadequate storage, transportation, and marketing facilities.

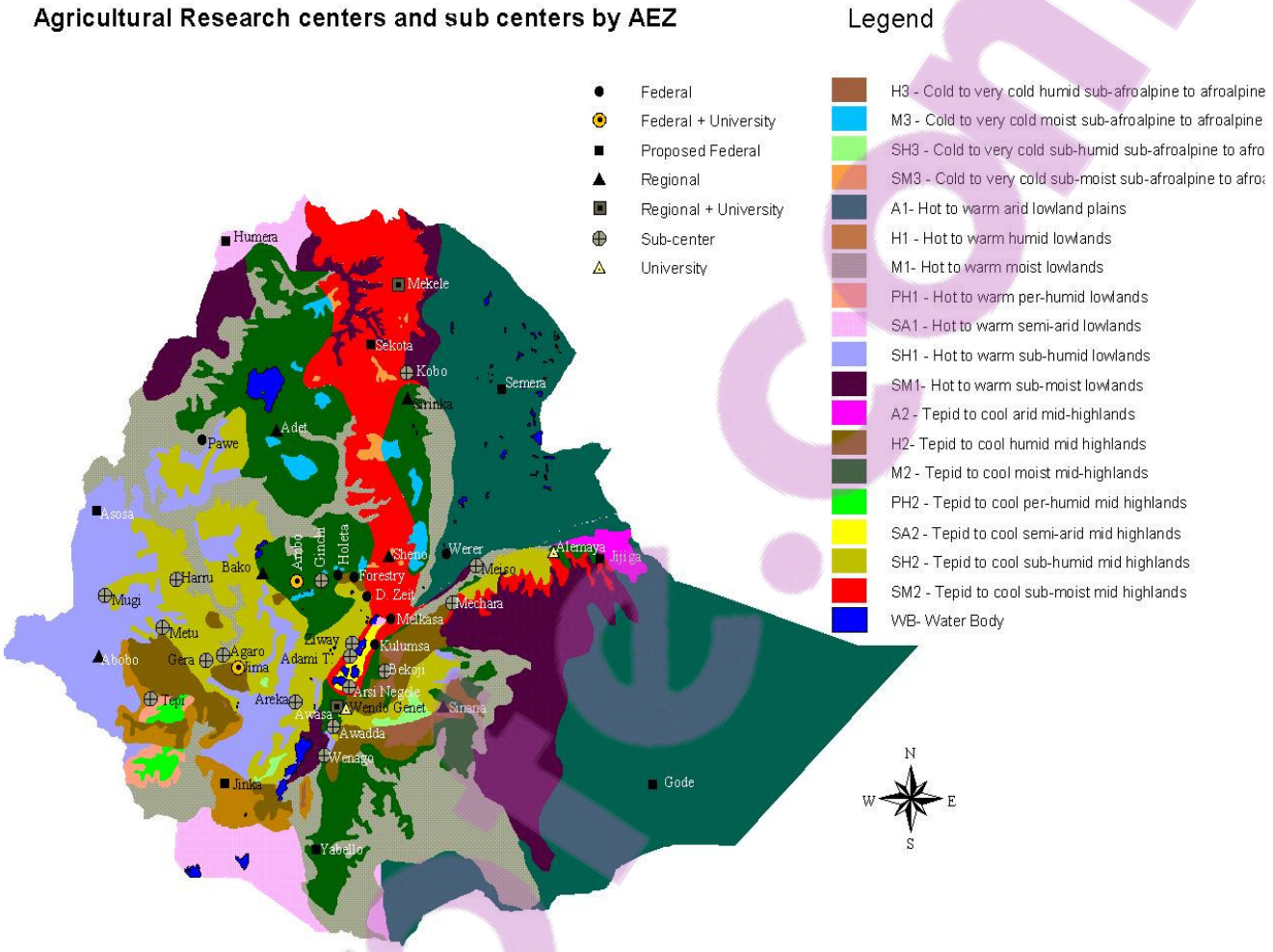

Figure 1.1. The major and sub agro-ecological zones of Ethiopia (EARO/ARTP, 1999)

Potato is exported to Djibouti and Somalia from the highlands of the eastern part of the country. There is a high demand and attractive prices for quality ware potatoes. Despite this great potential further expansion has been restricted due to the shortage of land as the highlands of the region are densely populated (land holding approximately 0.25 ha per farmer) and the majority of the land is used for cereals such as sorghum and maize production.

Among other environmental conditions, temperature and photoperiod are known to affect the various physiological processes of the potato plant. In general, potato prefers a cool climate for growth and development. Optimum temperatures for foliage growth and net photosynthesis are 15-25 ^{o}C, and 20 ^{o}C for tuberization. When the temperature is above 29 ^{o}C tuberization is inhibited, foliage growth is promoted and net photosynthesis and assimilate partitioning to the tubers are reduced (Gawronska *et al*., 1992; Hammes & De Jager, 1990; Levy, 1992; Menzel, 1980). The potato crop is a remarkable adaptable crop and with the development of modern cultivars and appropriate technologies, its production is being expanded in different parts of the world. However, its production in the hot tropical climates, i.e. regions with an altitude up to 1000 m, day length of approximately 12 h, minimum night temperature of 19-20 ºC, and maximum day temperature as high as 40 ºC (Accatino, 1981, as quoted by Ewing & Keller, 1983), has been restricted due to unfavourably high temperatures. Both soil and air temperatures are important in influencing the growth of the potato (Haverkort, 1978; Ewing & Keller, 1983). In Ethiopia about 35% of the available agricultural land is located in the semiarid region of the country where potato production has not been practiced due to unfavourably high temperatures throughout the year (EARO/ARTP, 1999).

The negative effect of high temperatures on tuber formation is believed to mediated through the production of high levels of endogenous gibberellins (GA) (Menzel, 1983) that is known to delay or inhibit tuberization (Abdella *et al*., 1995; Vreugdenhil & Sergeeva, 1999). The hormonal balance controlling potato tuberization can be altered using paclobutrazol (Simko, 1994). PBZ is a triazole plant growth regulator known to inhibit GA biosynthesis and abscisic acid (ABA) catabolism through its interference with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway (Rademacher, 1997).

To develop adaptable cultivars for the eastern parts of Ethiopia, the Potato Improvement Program of Alemaya University has been introducing potato germplasm from the International Potato Centre. Most of the genotypes bloom profusely and some of them set berries under the growing conditions of the highlands of Eastern Ethiopia. Information regarding the effect of flowering and berry set on growth, tuber yield and quality of potato is scanty.

Limitations to potato production include the tendency towards excessive vegetative growth instead of tuber growth in the lowlands, and profuse flowering and fruit formation in some of the promising cultivars in the highlands. If potato production can be expanded to the warm lowland areas of Ethiopia it can contribute significantly towards nutritional self-sufficiency in the production of food crops. Hence, the main objectives of the study were:

1. To investigate the response of potato grown under non-inductive greenhouse conditions to paclobutrazol so as to generate information for further field trials.
2. To investigate the responses of potato grown in the hot tropical lowlands of Eastern Ethiopia to paclobutrazol as a possible intervention to introduce potato culture to these marginal areas.
3. To investigate the effects of cultivar and flower and fruit development on the growth, tuber yield and quality of potato, and to devise chemical control measures to prevent berry set.

CHAPTER 2
LITERATURE REVIEW

The literature review focuses on three main themes of the thesis, namely sexual reproductive growth, tuberization and paclobutrazol. Specific topics are reviewed in the relevant chapters.

2.1 SEXUAL REPRODUCTIVE GROWTH

2.1.1 Flower

The potato inflorescence is single or compound cymes, and the number of flowers per inflorescence and per cyme depends on genotype, the environment and the position of the inflorescence in the shoot system (Almekinders & Struik, 1996). Inflorescences at higher positions are characterized by fewer flowers than ones at lower positions (Almekinders & Wiersema, 1991; Almekinders & Struik, 1994).

The corolla is five lobed and can be white, yellow, blue, purple or striped according to the variety. The calyx is tubular and lobed. Five stamens are borne on the corolla tube and the pistil consists of two carpels that form a two-locule ovary with a single style and stigma, and the flower produces no nectar (Smith, 1968).

2.1.2 Pattern of flowering

A potato plant developed from a seed tuber consists of one or more aboveground shoots. In determinate cultivars the growth of each stem is terminated by an inflorescence, but stem growth

may continue from lateral buds (Almekinders & Struik, 1994; Vos, 1995). The new branches will again terminate with an inflorescence, and this process can continues for several cycles.

2.1.3 Flowering response

It has been reported that *Solanum tuberosum ssp andigena* flowers regardless of day length, but does better under short days, while *Solanum tuberosum ssp tuberosum* usually does not flower under short days (Sadik, 1983). Long days hasten potato flower primordia initiation and development (Almekinders, 1992). Extending a 12-hour photoperiod with 4-hour incandescent light promoted flower production (Turner & Ewing, 1988).

Under natural light condition, increasing the temperature up to 28 ºC improved flower production (Marinus & Bodlaender, 1975). Plants grown at a night temperature of 20 ºC produced more flowers and bloomed on average eight days earlier than plants exposed to 10 ºC night temperature (Turner & Ewing, 1988). They also reported the existence of an interaction between photoperiod and night temperature. Longer photoperiods and warmer night temperature promoted flower production, by preventing flower bud abortion.

Growing potato plants in a greenhouse where photosynthetically active ration (PAR) was reduced by about 50% inhibited flower bud development, thereby completely suppressed flower production (Turner & Ewing, 1988). Calvert (1969) observed that reducing the level of irradiance increased tomato flower bud abortion indicating that the production and availability of adequate assimilates are crucial for flower bud development.

High levels of potassium, phosphorus, and nitrogen favour flowering in potato (Bolle-Jones, 1954). High levels of nitrogen fertilizer specifically promotes flowering, according to Bamberg & Hanneman (1988).

Long photoperiods (Almekinders, 1992), warm temperature (Turner & Ewing, 1988), and high nitrogen levels are inhibitory to tuberization. It has been proposed that the aforementioned factors by altering the hormonal balance delay tuber formation (Krauss, 1985; Wheeler *et al.*, 1986; Vandam *et al.*, 1996) and promote shoot growth, thereby stimulating flowering.

2.1.4 Fruit set

The berry of potato is spherical with a diameter of 1.2 to 1.9 cm and green or purplish green tinged with violet. It has two compartments and contains numerous small seeds ranging in number from 50 to 500 (Smith, 1968; CIP, 1983).

Fruit set often does not take place even when conditions are ideal for flowering (CIP, 1983). This seems to indicate that the conditions favouring flowering are not necessarily optimal for the processes of fruit development. Sadik (1983) reported that flower abscission may occur due to factors such as lack of insect pollinators, poor pollen viability, and too low temperatures for pollen germination and fertilization. He also indicated that abscission can result from a competition between developing fruit and tubers for limiting growth factors.

Almekinders *et al.* (1995) studied berry yield and seed production as influenced by flower positions and reported that mean berry weight, number of seeds per berry and 100 seed weight

decreased from the proximal to the distal flower position. Berries reach full development six weeks after fertilization (CIP, 1983).

2.1.5 Assimilate partitioning as affected by reproductive growth

Growth and development of different plant parts are affected by total assimilate production and partitioning among sink organs. Shoot and tuber growth are considered competing processes. Since the conventional potato propagation rely on seed tubers, less attention has been given to the effect of flowering and berry set on the growth of potato. Some researchers have studied the effects of flowering and berry formation on vegetative growth and tuber yield but the results are conflicting. Knight (1807) as quoted by Bartholdi (1940) believed that the failure of early potato cultivars to produce seed was due to tuber formation, indicating that early growth of tubers utilises materials necessary for floral and fruit development. He concluded that preventing the formation of tubers promotes the formation of numerous flowers and berries.

Abdel-Wahar & Miller (1963) impeded the downward translocation of assimilates by wire girdling, stem incision, and stolon pruning, and observed profuse flowering, indicating that assimilate availability strongly influences flowering in potato. Bartholdi (1940) using indeterminate potato varieties observed that the non-flowering plants produced the greatest weight of tops and tubers, suggesting that sexual reproductive growth reduces vegetative and tuber growth. The effect of flowering and berry formation on tuber yield in *Solanum demissum Lind*. were investigated by ProundFoot (1965). He observed that in five out of twelve fruiting plants, berry yield was higher than tuber yield, and reproductive growth significantly reduced tuber yield. Jansky & Thompson (1990) investigated the effect of flower removal on potato tuber yield. In one year, flower removal increased tuber yield of clone ND860-2 under irrigated and

dry land conditions. In the next year, however, flower removal affect tuber yield. They concluded that, the response to flower removal appears to be dependent on the environmental conditions.

There are some reports indicating that flowering and fruiting do not affect tuber yield. Observation on reciprocal crosses between *S. andigena* and *S. tuberosum* clones indicated that fruiting has no effect on tuber yield (Cubelios, 1973 as quoted by Haile-Micheal, 1973). Newman & Leonial (1918) as quoted by Haile-Micheal (1973) observed a positive association between vegetative growth, tuber yield, and seed production for one cultivar grown under different conditions. Haile-Micheal (1973) working with reciprocal crosses reported that some of the highest yielding genotypes did set fruit profusely with little effect on tuber yield. This was especially true if the plants were grown under favourable environmental and cultural conditions. He concluded that fruit set did not materially contribute to the difference in tuber yield observed in reciprocal crosses.

2.2 TUBERIZATION

Potato tubers are shortened and thickened modified stems that bear scale leaves (cataphylls) each with a bud in its axil (Cutter, 1978). The usual site of tuber formation is a stolon tip. Stolons (rhizomes) are diagravitropic stems with long internodes and scale leaves. They develop as branches from underground nodes and are terminated by a curved apical portion called a hook (Peterson *et al*., 1985). According to Plaisted (1957) stolon formation starts at the most basal nodes and progresses acropetally. Wurr (1977) investigated the pattern of stolon formation in three cultivars and found that about half of the stolons were formed at the most basal node, with roughly 10% of the remaining stolons at each of the next four higher

nodes. It has been reported that stolons formed first normally grow longer, are more likely to branch, and are preferential sites for tuber formation (Lovell & Booth, 1969; Struik & Van Voorst, 1986).

The potato plant is remarkable for its plasticity in organ development (Steward *et al*., 1981; Clowes & MacDonald, 1987). Tuber formation can occur on almost every bud of the plant including axillary buds (Ewing 1985) and inflorescence (Marinus, 1993). The signal for induction to tuberization is omnipresent (can be transported to every plant part) and can express itself in all buds (Struik *et al.*, 1999).

An understanding of potato tuberization is important and the time of tuber initiation in relation to other aspects of plant development plays a vital role in determining potential yield.

2.2.1 Tuberization stimulus

The existence of tuberizing stimulus synthesized in the leaves and translocated to the site of tuber initiation was proposed by Gregory (1956). The movement of the tuberization stimulus across a graft union (from the induced scion to the underground nodes of non-induced stock) was demonstrated by Gregory (1956) and Kumar & Wareing (1973). However, reciprocal grafts did not tuberize. Studies on inter stem grafts showed that the tuberizing stimulus is transported acropetally and basipetally (Kumar & Wareing, 1973). The nature of this transmissible signal is not well known, but it is suspected to be a hormone and may have more than one component (Jackson *et al.*, 1998). The involvement of phytochrome in the production of the transmissible signal(s) was demonstrated by a grafting experiment of Jackson *et al*. (1998). Wild-type *Solanum tuberosum* ssp *andigena* induced to tuberize under

long day by grafting on a shoot from antisense phytochrome B plants but not by grafting on another wild-type plants.

The formation of stolons and tubers takes place preferably underground although the tuberization stimulus may be present throughout the plant and affects morphological development (Ewing, 1997). Under inductive conditions, both the young and old leaves are capable of producing the stimulus (Hammes & Beyers, 1973).

2.2.2 Major changes during tuberization

Potato tuberization is a complex process involving anatomical, enzymatic, biochemical and hormonal changes leading to the differentiation of the stolon into a vegetative storage organ, the tuber (Xu *et al.*, 1998, Jackson, 1999; Fernie & Willmitzer, 2001).

Anatomical changes

It has been reported that transformation of stolon into tuber involves cell division, change in the direction and orientation of the microtubule, and cell enragement (Koda, 1997). During tuber initiation many changes have been documented to occur in stolon tips. Xu *et al*. (1998) observed cell division in the apical and subapical regions (up to approximately 5 mm from the apex) of non-swelling but elongating stolons. Upon tuber initiation, cessation of stolon growth coincides with the cessation of mitotic activity in the apical meristems (Xu *et al*., 1998). Both cell division and cell enlargement contribute to the development of tubers (Xu *et al.*, 1998).

Biochemical changes

Biochemical changes associated with tuberization have been investigated by several molecular biologists (Park 1990; Prat, *et al.*, 1990; Sanchez-Serrano & Et, 1990). Before any

sign of tuber initiation, stolon tips undergo a change that increases the accumulation of soluble carbon compounds and increase the conversion of these to insoluble compounds (Oparka & Davies, 1985). As the stolon tips begin to develop into tubers, the activity of GA-like compounds in the stolon tips decreases (Koda & Okazawa, 1983); accumulation of starch increases and concomitantly the levels of glucose and fructose decrease (Geigenberger *et al*., 1998; Struik *et al.*, 1999); and a significant increase in the concentration of a storage protein (patatin) is observed (Hendriks *et al.*, 1991; Suh *et al.*, 1991).

In most plants fixed carbon is transported in the form of sucrose (Kühn *et al.*, 1999). It has been proposed that the ability of an organ to metabolise sucrose is one of the determining factors in regulating sink strength (Sung & Black, 1989). Carbohydrates are imported into the growing stolon and tubers via the phloem, mainly in the form of sucrose (Struik *et al*., 1999). Elongating but non-tuberizing stolons exhibit high activity of invertase, while sucrose synthase is absent; however, upon tuber formation the activity of sucrose synthase drastically increases and the activity of invertase decreases (Ross *et al*., 1994; Appeldoorn *et al.* 1997). The rise in sucrose synthase activity is positively associated with the onset of starch and storage protein synthesis (Obata-Sasamoto & Suzuki, 1979) and sink strength (Hajirezaei, *et al.*, 2000). A change in hexose to sucrose ratio in favour of the latter is observed in the stolon tip (Davies, 1984). This is attributed to a significant decease in hexose content, especially fructose, possibly caused by a higher fructokinase than hexokinase activity in the developing tubers (Davies & Oparka, 1985; Gardner *et al.*, 1992; Renz & Stitt, 1993). As a result, the level of fructose in the developing tubers is much lower than in stolons (Ross *et al*., 1994; Appeldoorn *et al*., 1997; Vreugdenhil & Sergeeva, 1999). The activity of ADPglucose pyrophosphorylase that catalyses the conversion of Glucose-1-P into ADPGlucose significantly increases upon tuberization (Visser *et al.*, 1994; Appeldoorn *et al*., 1997).

2.2.3 Factors affecting tuberization

Genetic factors

Most wild *Solanum* species have a short day critical photoperiod for tuberization; and will become induced only if the photoperiod is less than 12 hours. This holds true for *Solanum tuberosum* ssp. *andigena*, which is adapted to the short days and cool temperatures of the Andean area (Amador *et al*., 2001). In contrast, *Solanum tuberosum* sub sp. can tuberize under longer photoperiod; it has a much longer critical photoperiod (Ewing, 1997). Genetic mapping of backcrosses between ssp *tuberosum* and wild species has revealed the presence of at least eleven genes responsible for tuberization under long photoperiods (Van den Berg *et al*., 1996). The existence of variation among genotypes with respect to photoperiod sensitivity has been reported by Ewing (1995). Cultivars differ not only as to the percentage of stolons that bear tubers, but also with respect to the pattern of tuberization at different nodes (Ewing, 1997).

Mother tuber

The size as well as the physiological condition of the mother tuber exerts a definite effect on the development of plants by affecting stolon and tuber formation (Van der Zaag & Van Loon, 1987). As the physiological age of the mother tuber increases induction to tuberize increases and its effect on the morphology of the plant resemble that of a short photoperiod. Planting physiologically older seed tubers results in smaller plants with more stems, and promotes earlier tuberization and earlier senescence (Ewing, 1997). Villafranca *et al*. (1998) from a kinetin-induced *in vitro* tuber formation study reported that early tuberization increased with physiological age of the mother tuber.

Environmental factors

The tuber forming sequence in *Solanum* species normally consists of stolon development followed by tuberization in sub apical region of the stolon (Booth, 1963). These processes are controlled by environmental factors, primarily temperature and photoperiod (Gregory, 1956; Salter, 1968).

Photoperiod and light quality

Tuberization of potato plants is strongly influenced by daylength. Induction to tuberize is promoted by short photoperiod (long dark period) and the signal is perceived in the leaves (Gregory, 1965). Interruption of the dark period with red light is more inhibitory to tuberization than other wavelengths, and the inhibitory effect of red light can be reversed by exposure to far-red radiation. This provides evidence for the involvement of a photoreceptor phytochrome in this response (Batutis & Ewing, 1982). Using an antisense approach in short day *Solanum tuberosum* sub sp *andigena,* Jackson *et al.* (1996) observed a reduced level of the expression of phytochrome B (PHYB) in transgenic plants. Consequently, transgenic plants became insensitive to photoperiodic changes and tuberized both under short day and long day conditions. This response suggested that PHYB exerts a negative control over tuberization of *andigena* under long photoperiods. Jackson *et al.* (1998) demonstrated that this photoreceptor controls the synthesis of the graft-transmissible inhibitory signal that is produced under long days, and which is absent or inactivated in the PHYB-antisense plants.

There is evidence indicating that GA is a component of an inhibitory signal and prevent tuberization under long days condition. Exogenous GA application inhibited tuber initiation (Xu *et al.*, 1998). High activity of GA-like compounds was detected in potato grown under non-inductive conditions (Vreugdenhil & Sergeeva, 1999) and reduced GA activity was

detected in leaves exposed to short days (Ewing, 1995). A dwarf mutant characterized by partial blocking of GA biosynthesis tuberized under short and long days (Van den Berg *et al*., 1995). Treating wild-type *andigena* spp plants with GA synthesis inhibitor, ancymidal, promoted tuberization under long days (Jackson & Prat, 1996). Like high temperatures, long photoperiod delays the onset of tuber growth and bulking (Vandam *et al.*, 1996). It decreases partitioning of assimilates to the tubers and increases partitioning to other parts of the plant (Wolf *et al*., 1990).

Temperature

Another important factor that exerts a major influence on tuberization is temperature. Generally, cool temperatures promote tuberization (Struik & Kerckhoffs, 1991; Vandam *et al*., 1996), and high temperatures are inhibitory for tuberization under both short and long photoperiods, albeit the degree of inhibition is greater under long days (Wheeler *et al.*, 1986). Both air and soil temperatures are important, cool air temperatures favour induction to tuberize (Gregory, 1956; Reynolds & Ewing, 1989), and high soil temperatures block the expression of the tuberization stimulus on the underground nodes (Reynolds & Ewing, 1989). There is an interaction between temperature and photoperiod. The higher the temperature the shorter the photoperiod required for a given genotype to tuberize (Snyder & Ewing, 1989).

At elevated temperatures foliage growth is promoted (Menzel, 1980), net photosynthesis decrease (Hammes & De Jager, 1990), assimilate partitioning to the tubers is reduced (Gawronska *et al.*, 1992) and dark respiration increases (Levy, 1992, Thornton *et al*., 1996). There is evidence that the inhibitory effects of high temperatures are mediated through the production of high levels of GA-like compounds known to inhibit tuber formation (Menzel,

1983). It has been suggested that high temperature exerts its influence on tuber formation by altering the balance between endogenous GA, cytokinins, and inhibitors (Menzel, 1985).

Irradiance

Similar to high temperatures and long photoperiod, low levels of irradiance during the day decrease the induction of tuberization (Bodlaender, 1963; Gregory, 1965; Demagante & Vander Zaag, 1988). Extension of the photoperiod with high level of irradiance (a mixture of fluorescent and incandescent lamps) was less inhibitory to tuberization than extending with low level of incandescent lamps only, may be due to its effect of extra assimilate production (Wheeler & Tibbitts, 1986; Lorenzen & Ewing, 1990).

Lowering the irradiance level decreases the partitioning of assimilates to the tubers (Gray & Holmes, 1970; Menzel, 1985). Shading experiment to reduce light level revealed that shading treatments had a pronounced effect in delaying tuberization, especially if applied after the onset of tuberization (Gray & Holmes, 1970; Sale 1976; Struik, 1986). Menzel (1985) reported that low irradiance increased the production of growth substances that inhibit tuber formation, and GA is the most likely candidate to play such a role.

Nitrogen nutrition

Induction to tuberize tends to decline with an increase in the level of nitrogen. Krauss (1985) demonstrated that tuberization could be manipulated by altering nitrogen supply to the plants. Continuous supply of 1 and 3 mM nitrogen completely inhibited tuber formation, while interrupting the nitrogen supply by keeping plants temporarily in a nitrogen free medium for 4 to 6 days promoted tuberization. He noted that repeated cycles of high nitrogen and nitrogen

withdrawal could result in the formation of "chain tubers", indicating that the level of nitrogen play a vital role in the control of tuber formation.

Increasing nitrogen fertilization enhanced partitioning of assimilates to the shoots rather than to the tubers (Biemond & Vos, 1992). Withholding nitrogen fertilization increased starch content of the leaves, increased the percentage export of assimilates from the leaves, and reduced the activity of sucrose phosphate synthase (Oparka *et al.*, 1987). Although how high nitrogen level inhibits tuberization is not well understood, there is a report indicating that nitrogen withdrawal affects the phytohormone balance in such a way that the level of GA decreases while increasing ABA level (Krauss, 1985). Koda & Okazawa (1983) suggested that the ratio between carbohydrate and nitrogen controls tuber formation. In an *in vitro* experiment, they observed that the inhibitory effect of higher nitrogen was observed only at 2% sucrose but not at a higher concentration.

Sucrose

There is evidence indicating high assimilate level is a contributing factor in induction besides hormonal factors. Gregory (1956) reported that for *in vitro* tuberization sucrose must be added to the growing medium. Sucrose is essential for *in vitro* tuber formation and its use is related with osmotic effect (Nawsheen, 2001). Oparka & Wright (1988) reported that starch synthesis is regulated by the osmolarity of the media. High sucrose level increases the osmotic potential of the media and enhances starch accumulation (Nawsheen, 2001). Khuri & Moorby (1995) proposed that high sucrose level provides a good carbon source that is easily assimilated and converted to starch for the microtuber growth and secures an uninterrupted synthesis of starch due to the higher osmotic potential provided by the excess sucrose. On the contrary, Perl *et*

al., (1991) pointed out that the requirement for high sucrose levels does not represent an osmotic effect or an energy demand but rather a signal for tuber formation.

Simko (1994) hypothesized that sucrose influence tuberization by altering the GA to promoter ratio in such a way that high exogenous sucrose supply causes the formation of excess UDPglucose which in turn increases conjugation of free GA. He also reported that application of glucose did not affect tuberization, because only a small amount of endogenous glucose is converted to sucrose. Cells that contain higher glucose, and lower sucrose concentration showed weak sucrose synthase activity (Sowokinos & Varns, 1992) and less UDPglucose was formed (Geigenberger & Stitt, 1993). Transgenic potato plants characterized by high level of sucrose (Müller-Röber *et al.*, 1992) and increased UDPglucose/hexose phosphate ratio (Jelitto *et al.*, 1992; Sonnewald, 1992) produced significantly higher number of tubers.

2.2.4 The role of plant hormones

Potato tuberization is a complex developmental process known to be influenced by genetic, environmental and physiological factors. Several plant hormones have been suggested to play a prominent role in the control of tuberization in potato (Vreugdenhil & Struik, 1989). Available evidence indicates that photoperiod, temperature, irradiance, nitrogen fertilization and physiological age of the mother tuber affect tuberization either directly or indirectly by mediating changes in hormone concentrations (Van der Zaag & Van Loon, 1987; Vreugdenhil & Struik, 1989; Ewing, 1990).

Gibberellin

The group of hormones most studied in relation to tuberization is the gibberellins (GA), and compelling evidences indicate that they play a vital role in tuberization. Exogenous application of GA reduced tuberization in intact plants, *in vitro* plantlets, and *in vitro* cultured excised sprouts (Menzel, 1980; Koda & Okazawa, 1983; Hussey & Stacey, 1984, Ewing, 1995). The application of GA-biosynthesis inhibitors promoted tuber initiation (Balamani & Poovaiah, 1985; Simko, 1994). Relatively high activity of GA-like compounds was detected in potato grown under non-inductive conditions, specifically under long photoperiods (Railton & Wareing, 1973), high temperature (Menzel, 1983), and high nitrogen fertilization (Krauss, 1985). On the contrary, under short day conditions GA biosynthesis is reduced (Amador *et al*., 2001). High levels of endogenous GA promote shoot growth (Menzel, 1980) and delay or inhibit tuberization (Abdella *et al*., 1995; Vandam *et al*., 1996), impede starch accumulation (Booth & Lovell, 1972; Paiva *et al*., 1983; Vreugdenhil & Sergeeva, 1999), inhibit the accumulation of patatin and other tuber specific proteins (Vreugdenhil & Sergeeva, 1999), and in combination with other inhibitors it regulates potato tuber dormancy (Hemberg, 1970).

GA inhibits tuberization and appears to play a role in the photoperiodic control of tuberization by preventing tuberization in long day (Jackson, 1999). The idea supported by enhanced tuberization of wild-type *Solanum tuberosum* sub sp. a*ndigena* treated with ancymidol, a GA biosynthesis inhibitor, under long day conditions (Jackson & Prat, 1996). A mutation that appears to block GA synthesis is associated with increased tuberization in potato (Bamberg & Hanneman, 1991, Van den Berg *et al.*, 1995). Amador *et al*. (2001) also suggested that GA is part of the inhibitory signal in potato tuberization under long days. The delaying or inhibitory effect of GA on tuberization may be partly attributed to its effect on carbohydrate metabolism especially sucrose utilization (Jackson, 1999). The involvement of GA in regulating the

pattern of assimilate partitioning was suggested by Yim *et al.*, (1997) who noted that high GA activity leads to higher carbohydrate allocation to the shoots, while low GA level resulted in more dry matter allocation to the roots. GA increases sink strength at the point of application (Mulligan & Patrick, 1979).

Cytokinins

Cytokinins belong to a class of plant hormones first noted as promoters of a cell division (Miller *et al.*, 1955). They are involved in various development processes including apical dominance, root formation, leaf senescence, stomatal behaviour, and chloroplast development (Mok, 1994). Cytokinins are necessary at the very early stage of tuber development, probably because of their vital role in stimulating cell division and radial cell growth (Ooms & Lenton, 1985; Gális *et al.*, 1995). *In vitro* induction of tuberization by exogenous application of cytokinin was reported by Palmer & Smith (1969). Menzel (1985) reported that benzyladenine treatment promoted tuberization in potato grown under high day/night temperatures (32/18 ºC). Exposing plants to inducing conditions (cool temperature and short photoperiod) temporarily increased leaf cytokinin content (Langille & Forsline, 1974). However, the concentration of cytokinin in stolon tips shows little increases until the tubers attain twice the diameter of the stolon (Koda & Okazawa, 1983). The major cytokinin isolated from potato leaves was identified as cis-zeatin riboside (Mauk & Langille, 1978). There are some indications that zeatin riboside (or other cytokinins) is at least partly involved in the tuberization stimulus (Vreugdenhil & Struik, 1989). Mauk & Langille (1978) also suspected that zeatin riboside may be the actual tuber-forming stimulus.

Recently, the involvement of cytokinins in regulating carbohydrate transport and metabolism, and in source-sink effects has drawn much attention (Roitsch & Ehneß, 2000). Kuiper (1993)

hypothesized that cytokinins are involved in regulation of the competition for assimilates and in the creation of sinks by regulating the expression of genes. Modification of the endogenous cytokinin level resulted in redistribution of assimilates in favour of the cytokinin-enriched axillary buds (Guivarc'h, *et al.*, 2002).

Auxins

The direct effect of auxins on tuberization is not yet well investigated. The available information show that auxin is involved in controlling apical dominance, and in combination with GA and cytokinins controls stolon orientation and growth (Ewing & Struik, 1992). Harmey *et al.* (1966) reported that IAA treatment promoted tuberization by inducing the formation of larger tubers at an early stage. High auxin content before tuber initiation and a subsequent decrease during tuber development was reported by Obata-Sasamoto & Suzuki (1979). The application of IAA in the tuber-inducing medium of *in vitro* plantlets led to earlier tuber initiation and produced smaller and sessile tubers (Xu *et al.*, 1998). In addition, they observed that application of IAA in a 1% sucrose medium totally blocked the growth of lateral buds of the cutting and this seems to indicate that IAA restricts elongation growth. Kumar & Wareing, (1972) speculated that IAA stimulates tuber formation by inhibiting stolon elongation and counteracting the effect of endogenous GA that promotes stolon formation and elongation.

Abscisic Acid

Conflicting results have been reported regarding the effects of abscisic acid (ABA) on tuberization. A stimulation of tuber formation in long-day-grown potato was observed in response to leaf applied ABA (El-Antably *et al.*, 1967). Wareing & Jennings (1980) reported that ABA promoted tuberization in leafless induced cuttings. Exogenous applied ABA

stimulated tuberization, reduced stolon length, increased tuber number, and induced the formation of sessile tubers (Menzel, 1980, Xu *et al.*, 1998). Furthermore, an increased ABA level under tuber-inducing conditions was reported by Krauss & Marschner (1982). On the contrary, the inhibitory effects of ABA on tuberization have been reported by Palmer & Smith (1969) and Hussey & Stacey (1984).

ABA is the most wide spread growth inhibitor in plants (Salisbury & Ross, 1992). Treatments with plant growth regulators that block endogenous GA synthesis promote tuberization in potato (Balamani & Poovaiah, 1985; Simko, 1994). This suggests that the naturally occurring tuberization stimulus contains inhibitors or antagonists of GA, and ABA is a likely candidate (Krauss & Marschner, 1982; Wareing & Jennings, 1980). Simko *et al.* (1996) reported a close association between the location of several genes controlling to tuberize under long photoperiod and genes for ABA levels.

Ethylene

The influence of ethylene on tuberization processes depends on the method of application and the type of tissue used (Stallknecht, 1985). The application of ethephon to a very old seed tuber causes a restoration of more normal sprout growth instead of the formation of sprout tubers directly at the eye. Higher GA activity was detected in the elongated sprouts than in the sprout tubers, and ethylene stimulated high GA activity that in turn inhibited tuberization (Dimalla & Van Staden, 1977).

The inhibitory effect of ethylene and promotion effect of ethylene antagonists in *in vitro* tuberization was reported by Vreugdenhil & Struik (1990). Chlorethylphosphonic acid (CEPA) inhibited tuber formation at low day/night temperatures (22/10ºC) according to

Menzel (1985). However, other studies on the effect of ethylene showed contradictory results. Garcia-Torres & Gomez-Campo (1972) reported more tubers on potato plants treated with ethrel than on untreated plants. Similarly, application of ethrel in the medium advanced tuberization and increased tuber number on excised potato sprouts cultured *in vitro* (Stallknecht & Farnsworth, 1982).

It is believed that hormones play a vital role in the communication of signals between plant organs. All classes of plant hormones have some effect on one or more aspects of the different steps leading to tuber formation (Vreugdenhil & Struik, 1989; Ewing & Struik, 1992; Ewing, 1995). The concept of a balance between hormones rather than the concentration of a single hormone as controlling mechanisms in tuber induction has received due consideration. Okazawa & Chapman (1962) suggested that the balance between inhibitory and promoting substances regulates tuber formation. Hammes & Nel (1975) also proposed that a balance between endogenous GA and tuber forming stimuli controls tuber formation; for tuberization to occur the GA must be below a threshold level.

2.3 PACLOBUTRAZOL

The use of chemical plant growth regulators to improve crop productivity has interested plant scientists for many years. Moreover, the recent development of highly active growth retardants has further enhanced the potential uses of chemical growth regulators. Among them, paclobutrazol (PBZ) is widely used. PBZ, a member of triazole plant growth regulator group, is a broad-spectrum GA biosynthesis inhibitor and used widely in agriculture (Davis & Curry, 1991).

2.3.1 Chemistry

PBZ ([(2R, 3R+2S, 3S)-1-(4-chloro-phenyl) 4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol]) has been developed as a plant growth regulator and is registered with trade names such as Bonzi, Clipper, Cultar, and Parsley. It belongs to the triazole compounds that are characterized by a ring structure containing three nitrogen atoms, chlorophenyl and carbon side chains (Fletcher *et al.*, 1986). Structurally, PBZ is a substituted triazole with two asymmetric carbon atoms (Fig. 2.1) and is produced as a mixture of 2R, 3R, and 2S, 3S enantiomers (Sugavanam, 1984, Hedden & Graebe, 1985).

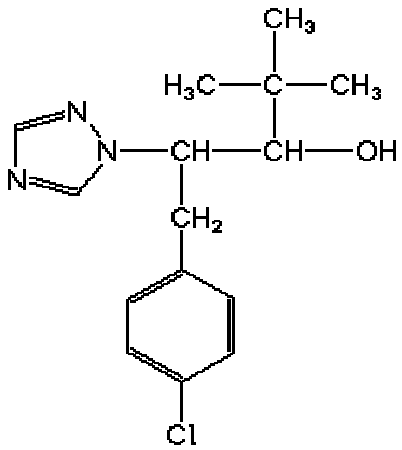

Figure 2.1 The structure of PBZ (http:/www.hclrss.demon.co.uk/ paclobutrazol.html)

2.3.2 Mode of action

Although the precise features of the molecular structure which confer plant growth regulatory activities are not well understood, it appears to be related to the stereochemical arrangement of the substituents on the carbon chain (Fletcher & Hofstra, 1988). There are indications that enantiomers having S configuration at the chiral carbon bearing the hydroxyl group are inhibitors of GA biosynthesis. In cell-free systems, the 2S and 3S enantiomers inhibited ent-kaurene oxidation more effectively than 2R and 3R forms (Hedden & Graebe, 1985). Roberts

& Mathews (1995) reported that resistance of *Chrysanthemum* plants to desiccation was associated with the activity of 2S and 3S enantiomers, presumably due to the inhibition of GA biosynthesis.

2.3.3 Translocation and chemical stability

It was previously believed that triazoles were primarily transported acropetally in the xylem (Davis *et al*., 1988). However, PBZ has been detected in xylem and phloem sap of castor bean (Witchard, 1997), pear (Browning *et al.*, 1992) and dessert pea (Hamid & Williams 1997) indicating that triazoles can be transported acropetally and basipetally. Although the metabolic fate of applied triazoles has not been investigated in detail most of them have a high chemical stability (Jung *et al*., 1986) and depending on the site of application tend to be metabolised slowly (Davis & Curry, 1991). Early & Martin (1988) observed more rapid PBZ metabolism in apple leaves than other plant parts, while Sterrett (1988) found little evidence for PBZ metabolism in apple seedlings. PBZ is comparatively more resistant to degradation than BAS 111 (Reed *et al.*, 1989).

2.3.4 Method of application

A simple, economical and efficient method of application capable of yielding consistent results is the top priority in the utilization of plant growth regulators for commercial purpose. Depending on plant species and concentration different responses have been observed for foliar and soil drenching of PBZ. PBZ spikes were more effective than drench applications in reducing shoot elongation of poinsettias (Newman & Tant, 1995). Drench application of PBZ was more effective in retarding the height of potted mussaenda than foliar spray (Cramer &

Bridgen, 1998). Foliar spray may not give uniform plant size modification if the coverage is inadequate (Barrett *et al.*, 1994). Generally, PBZ is more effective when applied to the growing media and application on the growing medium would give longer absorption time and more absorption of active ingredient than foliar spray. Moreover, drench application of PBZ may directly inhibit GA synthesis as roots synthesize large quantities of GA (Sopher *et al.*, 1999). In some cases, however, both drench and spike applications are effective in controlling plant growth with similar concentration (Barrett *et al.*, 1994).

2.3.5 Response of plants to PBZ

A. Plant hormone biosynthesis

Gibberellin

PBZ interferes with GA biosynthesis by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P450-dependent oxygenases (Izumi *et al.*, 1985; Graebe, 1987). However, the biosynthetic pathway from mevalonic acid to kaurene and from kaurenic acid to GA_{12} aldehyde is not affected (Izumi *et al.*, 1985). The inhibitory effects of PBZ on GA biosynthesis is further supported by the fact that treated plants have lower GA concentrations (Steffens *et al.*, 1992), and some effects of PBZ could be reversed by GA application (Cox 1991; Guoping, 1997; Gilley & Fletcher, 1998).

Abscisic Acid

Triazoles interfere with the different isoforms of kaurene oxidase, a cytochrome P-450 hydroxylase and prevent abscisic acid catabolism (Zeevaart *et al.*, 1990; Rademacher, 1997). Contradictory results have been reported for the effects of PBZ on ABA levels in plants.

Increased ABA levels in response to PBZ treatment have been reported in *Actinidia* (Tafazoli & Beyl, 1993) and jack pine (Marshall *et al.*, 2000). Since increases in ABA levels have been associated with plant stress protection, it is suggested that PBZ induced stress protection could be mediated at least partly through its effects on the level of ABA (Fletcher & Hofstra, 1988). On the contrary, PBZ treatment reduced the level of ABA in rice seedling (Izumi *et al.*, 1988). The magnitude of the inhibitory effect of PBZ on ABA levels is dependent on the length of time after application (Buta & Spaulding, 1991). These differential responses may be attributed to differences in growth conditions, application methods, plant species, developmental stages, and the type and concentration of triazoles used (Grossman, 1990; Buta & Spaulding, 1991).

Cytokinin

Cytokinins are synthesized in the roots and translocated acropetally to the shoots where they regulate both plant development and senescence (Letham & Palni, 1983; Binns, 1994). They are involved in the control of various plant developmental processes such as cell division, apical dominance, stomatal behaviour, root formation, leaf senescence, and chloroplast development (Mok, 1994). The involvement of cytokinin in carbohydrate transport and metabolism has been suggested by Roitsch & Ehneß (2000).

Zhu *et al.* (2004) observed an increase in the endogenous cytokinin (Zeatin) level in xylem sap of young apple trees in response to PBZ treatment. PBZ treatment delayed the onset of senescence in grapevine (Hunter & Proctor, 1992) and blueberry (Basiouny & Sass, 1993). It has been reported that cytokinin or chemicals like thidiazuron with cytokinin-like activity stimulate chlorophyll synthesis and retard senescence (Letham & Palni, 1983; Visser *et al.*,

1992) and thus PBZ induced physiological responses may be associated with increased cytokinin synthesis or prevention of its degradation.

B. Chlorophyll synthesis

PBZ treated plants have dark green foliage. This has been associated with increased chlorophyll content of the leaf tissue (Sopher *et al.*, 1999; Berova & Zlatev, 2000; Sebastian *et al.*, 2002) and more densely packed chloroplasts per unit leaf area due to reduced leaf expansion (Khalil, 1995). The increase in chlorophyll content may be ascribed to higher cytokinin content that is known to stimulate chlorophyll biosynthesis and/or reduced chlorophyll catabolism (Berova & Zlatev, 2000). Sopher *et al.* (1999) reported that PBZ increased chlorophyll levels both on fresh weight and leaf area bases. In several plant species PBZ treated leaves were retained longer and the onset of senescence considerably delayed (Hunter & Proctor, 1992; Basiouny & Sass, 1993). The senescence delaying activity may be related to the influence of PBZ on the endogenous cytokinin content (Fletcher *et al.*, 2000).

C. Rate of Photosynthesis

Contradictory reports have been published regarding the effects of PBZ on crop photosynthetic efficiency. PBZ has little direct effect on photosynthetic efficiency; however, indirectly by reducing leaf area it may reduce photosynthetic surface area and thereby reduce the whole-plant photosynthesis (Davis *et al.*, 1988). Rate of photosynthesis in rice was not affected by PBZ treatment (Yim *et al.*, 1997). Application of 250 and 500 mg PBZ per plant reduced leaf photosynthetic rate in sweet orange plants (Joseph & Yelenosky, 1992). On the contrary, there are reports indicating that PBZ enhances photosynthetic efficiency. PBZ

treatment increased productivity by enhancing photosynthesis efficiency in soybean (Sankhla *et al.*, 1985), rapeseed (Zhou & Xi, 1993), and tomato (Berova & Zlatev, 2000). Higher ribulose-1,5-biphosphate carboxylase activity and increased capacity for electron transport could be the reasons for enhanced photosynthesis after PBZ treatment (Archbold & Houtz, 1988; Joseph & Yelenosky, 1992; Van den Boogaard, 1994). Increased chlorophyll content in response to PBZ treatment may substantially contribute for enhanced photosynthetic rate because higher chlorophyll content is one of the main factors stimulating the rate of photosynthesis and biological productivity (Mojecka-Breova & Kerin, 1995; Berova & Zlatev, 2000).

D. Stress protection

PBZ increases tolerance of various plant species against several environmental stresses such as drought and temperature (Marshall *et al.*, 1991; Kraus & Fletcher, 1994; Marshall *et al.*, 2000; Zhu *et al.*, 2004). Proposed biochemical mechanisms of these protective effects include a shift in hormonal balance, decrease in endogenous GA levels and a transitory rise in ABA level (Masia *et al.*, 1994; Rademacher, 1997; Zhu *et al.*, 2004). PBZ increases the survival rate of plants under drought conditions through a number of physiological responses. A reduction in the rate of transpiration (due to reduction in leaf area), increased diffusive resistance, alleviating reduction in water potential, increased relative water content, less water use, and increased anti-oxidant activity are some of the reported responses (Marshall *et al.*, 1991; Eliasson *et al.*, 1994; Kraus & Fletcher, 1994, Zhu *et al.*, 2004). PBZ significantly decreased chilling injury in pepper fruit and cucumber seedlings (Whitaker & Wang, 1987; Lurie *et al.*, 1995), and this may be ascribed to in inhibition of chilling induced degradation of membrane lipids (Whitaker & Wang, 1987). PBZ induced chilling tolerance was also associated with change in antioxidant enzyme profiles and an increase in ABA level (Tafazoli

& Beyl, 1993; Pinhero *et al.*, 1997). PBZ protects plants from high temperature induced injuries (Kraus & Fletcher, 1994; Pinhero & Fletcher, 1994). Protection against high temperature stress is accompanied by the production of low molecular mass stress proteins (Larsen *et al.*, 1988) and the increase in the activity of antioxidant enzymes (Upadhyaya *et al.*, 1990; Kraus & Fletcher, 1994).

E. Morphological and anatomical changes

Shoot

Compared with other plant growth retardants triazoles are potent and required in small quantities to inhibit shoot growth (Davis *et al*., 1988). PBZ has been widely used to control the size of fruit trees and agronomic crops (Davis & Curry, 1991). The most noticeable effect of PBZ is internode compression resulting in compact and short plants (Berova & Zlatev, 2000; Terri & Millie, 2000; Sebastian *et al.*, 2002; Yeshitela *et al.*, 2004). Modification of shoot growth with the aid of PBZ may be helpful in maximizing return per unit land by allowing increased plant populations of the compact plants per unit land area.

Leaves

PBZ induces various leaf morphological and anatomical modifications depending on plant species, growth stage, rate and method of application. It reduces leaf area (Sebastian *et al.*, 2002; Yeshitela *et al.*, 2004), increases the thickness of the epicuticular wax layer (Jenks *et al*., 2001), increase size of a vascular bundles, epidermal, mesophyll and bundle sheath cells (Burrows *et al*., 1992; Sopher *et al.*, 1999). Depending on the species PBZ modulate leaf conductance, transpiration rate, and water use efficiency. In tomato PBZ enhanced rate of photosynthesis and slightly increased rate of transpiration along with a reduced stomatal

conductance in the third leaf while in the fifth leaf higher photosynthetic efficiency was accompanied by higher transpiration and stomatal conductance (Berova & Zlatev, 2000). Strawberry leaf diffusive conductance was increased by PBZ treatment when measured 12 months after application (Archbold & Houtz, 1988).

Stems

The reduction of plant height following PBZ treatment is accompanied by various anatomical modifications depending on species and concentration. Berova & Zlatev, (2000) observed increased radial extension in tomato stems, but stem diameter was reduced by 12-50% in citrus root stock seedlings (Yelenosky *et al.* 1995). McDaniel *et al.* (1990) reported that PBZ treatment of poinsettia resulted in weaker stems due to suppression of the thickening of cell wall of phloem fiber caps, decreased width of xylem ring, and restricting the differentiation of interfascicular supporting tissue. In *Chrysanthemum*, PBZ treatment resulted in thin stems with increased development of secondary xylem and a reduced number of sclerenchyma bundle caps (Burrows *et al.*, 1992). Aguirre & Blanco (1992) found that PBZ treatment resulted in a decreased proportion of xylem in peach shoots, with a corresponding increase in the amount of phloem and cortex. PBZ induced radial expansion in plant organs may be due to reduced endogenous GA levels. GA limits the extent of radial expansion of plant organs (Wenzel *et al.*, 2000). Barlow *et al.* (1991) observed a decreased axial growth and an increased radial expansion in GA deficient mutant tomato plants.

Roots

Depending on the plant species and the concentration applied, PBZ induces root anatomical and morphological modifications. It increased root diameter in *Chrysanthemum* by increasing the number of rows and diameter of cortical cells (Burrows *et al.*, 1992). Increased root

diameter in soybean due to an increase in the size of cortical parenchyma cells was reported by Barnes *et al.* (1989). PBZ inhibited primary root elongation of pea, while promoting radial expansion of the cells (Wang & Lin, 1992). Yim *et al.* (1997) reported that PBZ treated rice seedlings had higher root dry mass and greater ability to produce new roots. Enhanced adventitious root formation in English ivy (Geneve, 1990) and increased rooting ability of mung bean cuttings (Porlingis & Koukourikou-Petridou, 1996) have been observed in response to PBZ treatment. Improved root formation may be attributed to increased assimilate partitioning to the roots due to reduced demand in the shoot (Symons *et al.*, 1990).

By influencing shoot and root morphology PBZ alter mineral uptake, although the effects are not consistent and well investigated. Yelenosky *et al.* (1995) reported that leaves from PBZ treated citrus seedlings had higher concentrations of N, Ca, B, and Fe. In apple seedlings, PBZ increased the foliar concentration of N, P, K, Ca, Mg, Mn, B, and Zn without affecting the concentration of Fe, Si and Pb (Wang *et al.,* 1985), while Wieland & Wample (1985) found PBZ treatment did not affect the concentration of N, P, K, and Mg in apple leaves. Steffens *et al.* (1985) reported that apple fruit mineral composition was unaffected by PBZ treatment. Recently, Yeshitela *et al*. (2004) reported that PBZ increased mango leaf Mg, Cu, Zn, and Fe content without affecting the concentration of N, P, K, and Ca.

F. Assimilate partitioning

Sink regulation of photosynthesis is a well-accepted concept, possibly explaining the coordination of assimilate production and utilization (Stitt *et al.*, 1990). Assimilate partitioning to the different sinks may be controlled by environmentally regulated, hormonal balances (Almekinders & Struik, 1996). PBZ treatment increase the root-to-shoot ratio

(Pinhero & Fletcher, 1994; Yim *et al.*, 1997), increase partitioning of assimilates to economically important plant parts such as bulbs (Le Guen-Le Saos *et al.*, 2002, De Resende & De Souza, 2002) and tubers (Balamani & Poovaiah, 1985; Pelacho *et al.*, 1994; Simko, 1994). PBZ inhibits GA biosynthesis and subsequently modulates hormonal balance and thereby influences the pattern of assimilate production and allocation. The involvement of GA in regulating the pattern of assimilate partitioning was suggested by Yim *et al.* (1997). He noted that high GA level leads to a higher carbohydrate allocation to the shoots, where as low GA level resulted in more dry matter allocation to the roots.

CHAPTER 3

RESPONSE OF POTATO GROWN UNDER NON-INDUCTIVE GREENHOUSE CONDITIONS TO PACLOBUTRAZOL: SHOOT GROWTH, CHLOROPHYLL CONTENT, NET PHOTOSYNTHESIS, ASSIMILATE PARTITIONING, TUBER YIELD, QUALITY AND DORMANCY

3.1 ABSTRACT

The effect of foliar and soil applied PBZ on potato were examined under non-inductive conditions in a greenhouse. Single stemmed plants of the cultivar BP1 were grown at 35 (±2)/20 (±2) ºC day/night temperatures, relative humidity of 60%, and a 16h photoperiod. Twenty-eight days after transplanting PBZ was applied as a foliar spray or soil drench at rates of 0, 45.0, 67.5, and 90.0 mg active ingredient PBZ per plant. Regardless of the method of application, PBZ increased chlorophyll *a* and *b* content of the leaf tissue, delayed physiological maturity, and increased tuber fresh mass, dry matter content, specific gravity, and dormancy period of the tubers. PBZ reduced the number of tubers per plant. A significant interaction between rates and methods of PBZ application were observed with respect to plant height and tuber crude protein content. Foliar application resulted in a higher rate of photosynthesis than the soil drench. PBZ significantly reduced total leaf area and increased assimilate partitioning to the tubers. The study clearly showed that PBZ is effective to suppress excessive vegetative growth, favour assimilation to the tubers, increase tuber yield, improve tuber quality and extend tuber dormancy of potato grown in high temperatures and long photoperiods.

Keywords: Crude protein; gibberellin; high temperature; long photoperiod; paclobutrazol

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3.2 INTRODUCTION

High temperature is an important factor limiting potato production in some areas of the world (Morpurgo & Ortiz, 1988). The optimum temperatures for foliage growth and net photosynthesis are 20 - 25 ºC and 16 - 25 ºC, respectively. Low mean temperatures (15-19 °C) and short photoperiods (12 h) are favourable for tuberization and early tuber growth (Vandam *et al*., 1996). High temperatures inhibit tuberization in both short and long day conditions, but especially under long photoperiods (Jackson, 1999).

The carbon budget for potatoes developed by Leach *et al*. (1982) indicates that plant growth rate is strongly related to net photosynthesis and dark respiration. At elevated temperatures, foliage growth is promoted, rate of photosynthesis declines rapidly, assimilate partitioning to the tubers is reduced and dark respiration increases (Thornton *et al*., 1996). Tuber growth is completely inhibited at 29 ºC, above which point the carbohydrate consumed by respiration exceeds that produced by photosynthesis according to Levy (1992). Like high temperatures, long photoperiods also decrease partitioning of assimilates to the tubers and increase partitioning to other parts of the plant (Wolf *et al*., 1990).

Potatoes grown under high temperatures or long photoperiods are characterized by taller plants with longer internodes, increased leaf and stem growth, lower leaf: stem ratio, shorter and narrower leaves with smaller leaflets, and less assimilates partitioned to the tubers (Ben Khedher & Ewing, 1985; Manrique, 1989; Struik *et al*., 1989).

Induction to tuberization is promoted by short days, more specifically by long nights (Gregory, 1965) and cool temperatures (Ewing, 1981). Under such conditions a transmissible signal is

activated that triggers cell division and elongation in the sub-apical region of the stolons to produce tuber initials (Xu *et al*., 1998; Amador *et al*., 2001). In this signal transduction pathway, the perception of appropriate environmental cues occurs in the leaves and is mediated by phytochrome and GA (Van den Berg *et al*., 1995; Jackson & Prat, 1996).

Amador *et al*. (2001) reported that endogenous GA is a component of the inhibitory signal in potato tuberization under long days. Previous studies on GA showed that the levels of GA-like activity decrease in leaves of potato upon transfer from long day to short day conditions (Railton & Wareing, 1973). Under short day conditions GA biosynthesis is reduced (Amador *et al*., 2001). Van den Berg *et al*. (1995) reported that a dwarf potato mutant tuberized under long days due to the incorporation of a gene that partially blocks the conversion of 13-hydroxylation of GA_{12}-aldahyde to $GA_{53,}$ and treatment with GA biosynthesis inhibitors enhance tuberization in *andigena* spp. under long day conditions (Jackson & Prat, 1996).

Potato plants grown under non-inductive conditions are characterized by high levels of endogenous GA (Vreugdenhil & Sergeeva, 1999) that promotes shoot growth (Menzel, 1980) and delays or inhibits tuberization (Abdella *et al*., 1995; Vandam *et al*., 1996). In addition, accumulation of GA in tuber tissue can specifically impede starch accumulation (Booth & Lovell, 1972; Paiva *et al*., 1983; Vreugdenhil & Sergeeva, 1999), inhibits the accumulation of patatin and other tuber specific proteins (Vreugdenhil & Sergeeva, 1999), and in combination with other inhibitors it regulates potato tuber dormancy (Hemberg, 1970).

The hormonal balance controlling potato tuberization can be altered using GA biosynthesis inhibitors such as 2-chloroethyl trimethyl ammonium chloride (CCC) (Menzel, 1980), B 995 (Bodlaender & Algra, 1966), and PBZ (Simko, 1994). PBZ is a triazole plant growth regulator

known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway (Rademacher, 1997). Interference with the different isoforms of this enzyme could lead to inhibition of GA biosynthesis and abscisic acid (ABA) catabolism. In addition, it induces shoot growth reduction (Terri & Millie, 2000; Sebastian *et al*., 2002), enhances chlorophyll synthesis (Sebastian *et al*., 2002), delays leaf senescence (Davis & Curry, 1991) and increases assimilate partitioning to the underground parts (Balamani & Poovaiah, 1985; Davis & Curry, 1991; Bandara & Tanino, 1995; De Resende & De Souza, 2002).

It is postulated that PBZ blocks GA biosynthesis in potato plants grown under non-inductive growing conditions and modifies its growth to increase the productivity of the crop. Accordingly, the effects of foliar and soil applied PBZ on shoot growth, leaf chlorophyll content, assimilate production and allocation, tuber yield, and quality, and tuber dormancy period of potato grown under conditions of high temperatures and long photoperiod were investigated. The ultimate objective being to generate information to improve potato production in marginal areas where high temperatures and/or long photoperiods are limiting factors.

3.3 MATERIALS AND METHODS

3.3.1 Plant culture

Two experiments with similar procedures and treatments were conducted in 2002 on the experimental farm of the University of Pretoria, South Africa. Potato tubers of a medium maturing commercially cultivated variety BP1 were allowed to sprout, and seed cores of approximately 15 g containing the apical sprout were excised. Seed pieces were planted in crates

with vermiculite and kept in a growth chamber at 35/20 ^{o}C day/night temperatures and a 16h photoperiod. A week after emergence, uniform plants were transplanted to 5-liter plastic pots filled with sand and coconut coir (50:50 by volume) and grown in a greenhouse at 35 (±2)/20 (±2) ^{o}C day/night temperatures, an average relative humidity of 60%, and a 16h photoperiod. The photoperiod was extended using a combination of Sylvania fluorescent tubes and incandescent lamps (PAR: 10 µmol m^{-2} s^{-1}). In both experiments, the pots were arranged in a randomised complete block design with three replications and each replicate contained seven pots per treatment. Plants were fertilized with a standard Hoagland solution and watered regularly to avoid water stress.

3.3.2 Treatments

Twenty-eight days after planting (early stolon initiation) the plants were treated with PBZ at rates of 0, 45.0, 67.5 and 90.0 mg active ingredient (a.i.) per plant as a foliar spray or soil drench using the Cultar formulation (250 g a.i. PBZ per liter, Zeneca Agrochemicals SA (PTY.) LTD., South Africa). For the foliar treatment, the solution was applied as a fine spray using an atomizer. The drench solution was applied to the substrate around the base of the plants. The control plants were treated with distilled water.

3.3.3. Data recorded

Net photosynthesis and chlorophyll content

Two weeks after treatment the rate of photosynthesis was measured using a portable photosynthesis system (CIRAS-1, 1998, UK), and leaf chlorophyll content was determined. From each treatment, three plants were randomly selected and rate of photosynthesis was

measured on the terminal leaflet of three fully expanded younger leaves. The photon flux density incident at the level of the leaf in the cuvette was 1050-1220 $\mu molm^{-2}s^{-1}$ (PAR). Average saturated vapour pressure of water at cuvette temperature was 34.5 mbar and vapour pressure deficit of the air in the course of measurements was 6.05 mbar. To determine the concentrations of chlorophyll *a* and *b* spectrophotometer (Pharmacia LKB, Ultrospec III) readings of the density of 80% acetone chlorophyll extracts were taken at 663 and 645 nm and their respective values were assessed using the specific absorption coefficients given by MacKinney (1941).

Assimilate partitioning and total leaf area

Two, four, six, and eight weeks after treatment application one pot per treatment was harvested and separated into leaves, stems, tubers, and roots and stolons. Leaf area was measured with a LI-3000 leaf area meter (LI-Inc, Lincoln, Nebraska, USA) and the plant tissues oven dried at 72 °C to a constant mass. Dry matter partitioning was determined from the dry mass of individual plant parts as a percentage of total plant dry mass.

Plant height, senescence, tuber fresh mass and number

Plant height refers to the length from the base of the stem to shoot apex. Plants were regarded as physiologically mature when 50% of the leaves had senesced. Tuber fresh mass and numbers represent the average tuber mass and count of three plants at the time of final harvest.

Quality assessment

At harvest a representative tuber sample from each treatment group was taken and washed. Tuber specific gravity was determined by weighing in air and under water (Murphy & Goven, 1959). For dry matter content determination, the samples were chopped and dried at a

temperature of 60 ºC for 15h, and followed by 105 ºC for 3h. Dry matter content of the tubers is the ratio between dry and fresh mass. Samples dried at 60 ºC were analysed for total nitrogen (Macro-Kjeldahl method, AOAC, 1984), and tuber crude protein content estimated by multiplying total nitrogen content by a conversion factor of 6.25 (Van Gelder, 1981).

Dormancy

To determine the effect of PBZ on dormancy, six healthy tubers per treatment were selected at the final harvest and labelled. Each treatment was replicated three times and samples were randomly distributed on shelves in a dark room. The dormancy of a particular tuber was deemed to have ended when at least one 2mm long sprout was present (Bandara & Tanino, 1995).

3.3.4 Data analysis

The analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C, 1991). Combined analysis of variance did not shown significant treatments by experiment interactions. Hence, for all of the parameters considered, the data of the two experiments were combined. Means were compared using the least significant difference (LSD) test at 1% probability level. Correlations between parameters were computed when applicable.

3.4 RESULTS

PBZ treatment considerably reduced leaf area per plant. Irrespective of the rate of application the leaf area of PBZ treated plants were typically 50% smaller than the control at two, four, and six weeks after application (Figure 3.1). Plant height was influenced by the interaction effect of rate and method of PBZ application (Table 3.1). Foliar spray of 45 and 67.5 or 90 mg a.i. PBZ per

plant reduced plant height by about 35 and 46 % while soil drenching of the same concentration brought about 54 and 63% height reduction compared to the control, respectively.

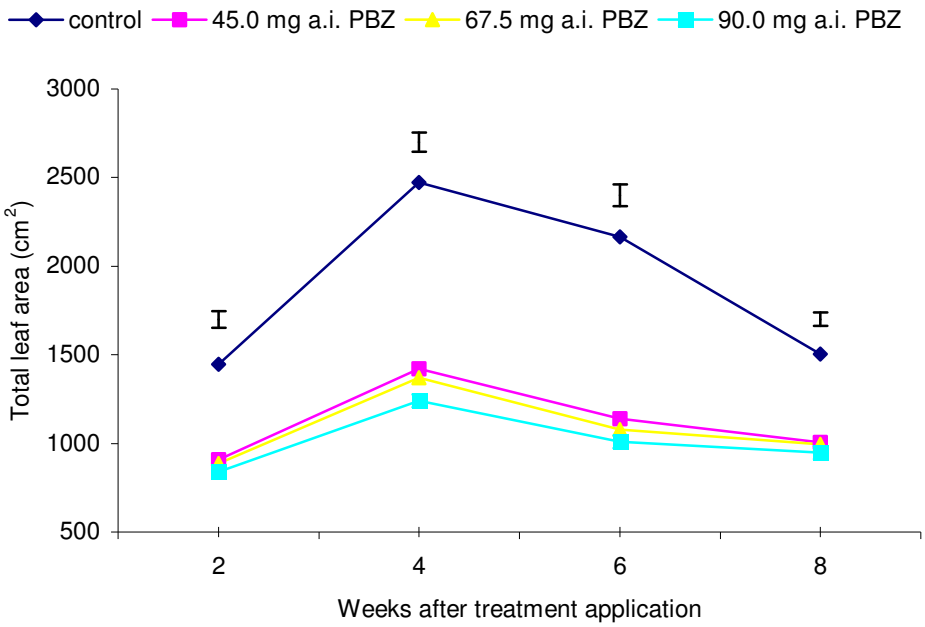

Figure 3.1 Total leaf area per plant as influenced by different rates of PBZ. The vertical bars represent least significant differences at P < 0.01

Table 3.1 Plant height of potato as affected by method and rate of PBZ application

PBZ rate (mg a.i. plant^{-1})	Plant height (cm)	
	Foliar spray	Soil drench
0 (control)	58.16a	59.32a
45.0	37.96b	27.45de
67.5	33.35c	23.78ef
90.0	29.53cd	20.52f
SEM	1.15	

SEM: standard error of the mean.

Means within columns and rows sharing the same letters are not significantly different (P < 0.01).

Regardless of the method of application, PBZ increased chlorophyll *a* and *b* content of the leaf tissue (Table 3.2). The highest chlorophyll *a* (0.86 mg g^{-1} FW) and chlorophyll *b* (0.31 mg g^{-1}

FW) values were obtained at the highest rate of PBZ application. An increase in chlorophyll *a* and chlorophyll *b* were observed with increasing rate of application.

Physiological maturity was influenced by the rate of PBZ application (Table 3.2). The treated plants retained photosynthetically active leaves longer and delayed the date to 50% senescence by approximately 20 days compared to the control.

Table 3.2 Chlorophyll *a* and *b* contents of leaf tissue, leaf net photosynthesis and days to physiological maturity as influenced by method and rate of PBZ application

Treatment	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Leaf net photosynthesis ($\mu molm^{-2}s^{-1}$)	Days to physiological maturity
Foliar spray	0.69a	0.22a	10.50b	96.13a
Soil drench	0.71a	0.20a	9.54a	96.00a
SEM	0.02	0.02	0.29	0.41
0 (control)	0.50c	0.14b	6.79b	81.48c
45.0 (mg a.i. $plant^{-1}$)	0.67b	0.15b	10.74a	99.71b
67.5 (mg a.i. $plant^{-1}$)	0.78ab	0.23ab	11.65a	100.70ab
90.0 (mg a.i. $plant^{-1}$)	0.86a	0.31a	10.91a	102.39a
SEM	0.03	0.03	0.41	0.58

SEM: standard error of the mean.

FW: Fresh weight.

Means of the same main effect within the same column sharing the same letters are not significantly different ($P < 0.01$).

Leaf net photosynthesis was significantly affected by rate and method of PBZ application (Table 3.2). The highest net photosynthetic rate was observed in plants treated with 67.5 mg a.i. PBZ per plant. Foliar treated plants showed higher net photosynthetic rate than soil drench treated plants.

PBZ affected the pattern of assimilate allocation to the different plant parts (Table 3.3). PBZ greatly reduced the partitioning of assimilate to the leaves, stems, and roots and stolons, and increased the partitioning to the tubers compared to the control, at all harvesting stages. There was no consistency in the effects of methods of application on the pattern of assimilate production and allocation.

Table 3.3 Dry matter distribution (% of the total dry mass) among plant organs of potato as influenced by rate and method of PBZ application

Main effect	Treatment	Leaf	Stem	Root & stolon	Tuber	Leaf	Stem	Root & stolon	Tuber
		---------- **Harvest I** ----------				---------- **Harvest II** ----------			
Method	Foliar spray	41.32a	23.59a	19.16a	15.93b	35.54a	23.93a	16.19b	24.34a
	Soil drench	41.83a	23.73a	18.06b	17.38a	36.00a	23.95a	17.48a	22.57b
	SEM	0.48	0.34	0.31	0.29	0.33	0.31	0.29	0.19
Rate	0 (control)	45.79a	33.18a	19.21a	1.82c	45.50a	27.53a	18.04a	8.93 c
	45.0 (mg)	39.65b	21.15b	19.12a	20.08b	33.56b	23.14b	15.88b	27.42b
	67.5 (mg)	39.40b	20.08b	18.81ab	21.71a	32.02b	22.57b	16.54ab	28.86a
	90.0 (mg)	39.45b	20.22b	17.30b	23.02a	32.01b	22.52b	16.86ab	28.61a
	SEM	0.68	0.48	0.43	0.41	0.46	0.44	0.41	0.37
		---------- **Harvest III** ----------				---------- **Harvest IV** ----------			
Method	Foliar spray	35.52a	25.71b	14.71a	24.06a	34.60a	24.58b	12.98a	27.84a
	Soil drench	33.20b	27.76a	15.54b	23.51a	32.93a	26.95a	12.93a	27.20a
	SEM	0.29	0.36	0.14	0.22	0.30	0.33	0.19	0.22
Rate	0 (control)	40.30a	28.72a	18.53a	12.44c	41.07a	28.74a	15.50a	14.82c
	45.0 (mg)	31.90b	27.49a	14.59b	26.02b	31.00b	26.24b	12.20b	30.29b
	67.5 (mg)	31.78b	25.48b	14.16b	28.58a	30.80b	24.24c	12.75b	32.52a
	90.0 (mg)	33.45c	25.25b	13.22c	28.08a	32.18b	23.85c	11.47b	32.44a
	SEM	0.40	0.50	0.21	0.32	0.42	0.47	0.26	0.32

SEM: standard error of the mean.

Harvests I, II, III and IV done two, four, six and eight weeks after treatment application.

Means of the same main effect within the same column sharing the same letters are not significantly different ($P < 0.01$).

Regardless of the method of application, PBZ treatment increased tuber fresh mass, dry matter content, and specific gravity but reduced tuber numbers (Table 3.4). Tuber fresh mass per plant increased from 71.9 g (control) to 155.6 g in response to application of 67.5 mg a.i. PBZ per plant. Increasing the rate of PBZ resulted in a concomitant reduction in tuber number. Averaged over the methods of application, treatment with 45.0, 67.5 and 90 mg a.i. PBZ decreased tuber number by 23, 33 and 43%, respectively, as compared to the control. PBZ boosted dry matter content and specific gravity by an average of 20% and 1.4%, respectively compared to the control. There was a tendency towards reduced tuber fresh mass, dry matter content and specific gravity at the higher rate of PBZ application.

Table 3.4 Tuber fresh mass, number, dry matter, specific gravity, and dormancy period as influenced by rates of PBZ application

PBZ rate (mg a.i. plant^{-1})	Tuber fresh mass (g pot $^{-1}$)	Tuber number pot $^{-1}$	Dry matter (%)	Specific gravity (g cm^{-3})	Dormancy period (days)
0 (control)	71.9c	10.47a	16.00b	1.048b	13.84b
45.0	151.5b	8.05b	18.90a	1.061a	42.30a
67.5	155.6a	7.00c	19.82a	1.065a	43.92a
90.0	141.2a	6.01d	18.90a	1.061a	44.08a
SEM	5.0	0.20	0.26	0.001	0.53

SEM: standard error of the mean.

Means within the same column sharing the same letters are not significantly different ($P < 0.01$).

PBZ extended the tuber dormancy period (Table 3.4, Figure 3.2). As the plants were grown under constant high day and night temperatures the tubers had a relatively short dormancy period. Irrespective of the concentration, PBZ extended the dormancy period by nearly a month as compared to the control.

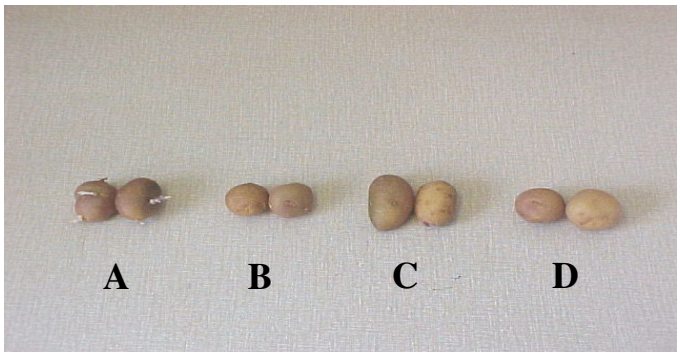

Figure 3.2 Dormancy characteristics of the control and PBZ treated potato tubers stored in a dark room, a month after harvesting. A = tubers from untreated plants (control), B = tubers from plants treated with 45 mg a.i. PBZ, C = tubers from plant treated with 67.5 mg a.i. PBZ, and D = tubers from plant treated with 90 mg a.i. PBZ

A significant interaction between rate and method of application was observed for tuber crude protein content (Table 3.5). Applying 45.0 or 67.5 mg a.i PBZ as a foliar spray gave the highest crude protein content, while drench application of 67.5 or 90.0 mg a.i. PBZ resulted in the highest crude protein content.

Table 3.5 Tuber crude protein content as influenced by rate and method of PBZ application

PBZ rate (mg a.i. plant^{-1})	Crude protein (%)	
	Foliar	Soil drench
0 (control)	2.09de	1.96e
45.0	2.35bc	2.22cd
67.5	2.28bc	2.24ab
90.0	2.08de	2.54a
SEM	0.04	

SEM: standard error of the mean.

Means within column and row sharing the same letters are not significantly different ($P < 0.01$).

3.5 DISCUSSION

Triazoles are potent plant growth regulators that inhibit shoot growth at low concentrations. PBZ effectively suppresses growth in a wide range of plant species and the treated plants tend to be darker green, shorter and more compact in appearance (Kamoutsis *et al.,* 1999; Terri & Millie, 2000; Sebastian *et al*., 2002). Shoot growth reduction occurs primarily due to decreased internode length, and the effective dose varies with species and cultivar (Davis & Curry, 1991). The most noticeable potato growth response to PBZ treatment was the reduction in shoot growth. As a result, treated plants were short and compact. This response could be attributed to reduction in total leaf area and stem elongation (height). Haughan *et al*. (1989) reported reduced cell proliferation due to PBZ treatment that may probably be responsible for restricted shoot growth.

Previous investigations on different crops showed that the foliage of PBZ treated plants typically exhibits an intense dark green colour due to enhanced chlorophyll synthesis (Sebastian *et al*., 2002) and/or more densely packed chloroplasts per unit leaf area (Khalil, 1995). A similar explanation is suggested for the increased chlorophyll *a* and *b* contents reflected in Table 3.2. The observed negative correlations between total leaf area and chlorophyll *a* content (r = - 0.91*) as well as total leaf area and chlorophyll *b* content (r = - 0.65) indicate that reduction in leaf area was associated with the higher chlorophyll *a* and chlorophyll *b* concentrations. Balamani & Poovaiah (1985) and Bandara & Tanino (1995) also observed an increase in chlorophyll content of potato leaves in response to PBZ treatment. The higher chlorophyll content and delayed senescence of PBZ treated potato leaves may be related to the influence of PBZ on the endogenous cytokinin content. It has been proposed that PBZ stimulates cytokinin synthesis that enhances chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher *et al*., 1982). The use of GA biosynthesis inhibitors increased cytokinin

content in rice (Izumi *et al*., 1988), soybean (Grossman, 1992) and *Dianthus caryophyllus* (Sebastian *et al*., 2002). Previous investigations revealed that the onset of senescence in several plant species was considerably delayed by triazoles (Davis & Curry, 1991; Binns, 1994).

PBZ increased the rate of net leaf photosynthesis (Table 3.2). This could be attributed to the higher chlorophyll content and earlier tuberization in response to the PBZ treatment. Increased net photosynthesis in response to PBZ has been reported in soybean (Sankhla *et al*., 1985) and rape (Zhou & Xi, 1993). Compelling evidence exists that application of GA reduces tuberization in potato, and GA biosynthesis inhibitors promote tuberization (Balamani & Poovaiah, 1985; Simko, 1991; Langille & Helper, 1992; Bandara & Tanino, 1995). Although it is difficult to examine the rate of photosynthesis as a separate phenomenon, numerous reports in various crops have shown that increased sink demand results in increased source output (net CO_2 fixation); and decreased sink demand decreased source output (Geiger, 1976; Hall & Milthorpe, 1978; Peet & Kramer, 1980). Rapid tuber growth increased the rate of net photosynthesis and enhanced translocation of photosynthates to the tubers (Dwelle *et al*., 1981a, Moorby, 1968). Alternatively, removal of rapidly growing tuber sinks led to a marked depression in photosynthetic efficiency due to an imbalance between source and sink (Nosberger & Humphries, 1965).

Dry matter partitioning was affected by PBZ treatment and at all harvesting stages tubers were the dominant sinks. This dominance might be associated with PBZ stimulated low GA level in the tuber tissue that increases tuber sink activity. Elevated temperatures and/or long days stimulate GA biosynthesis and thereby encourage top growth (Menzel, 1981; Vreugdenhil & Sergeeva, 1999). Exogenous GA application inhibited tuber formation; decreased sink strength of tubers and encouraged shoot and stolon growth (Menzel, 1980; Mares *et al*., 1981; Vreugdenhil & Struik, 1989). Similar reports have been published indicating that high

temperatures decrease tuber growth rate, decrease the partitioning of assimilates to the tubers and increase the amount allocated to other parts of the plant (Menzel, 1980; Struik *et al*., 1989; Vandam *et al*., 1996).

The PBZ treatments considerably increased tuber yield (Table 3.4) and this may be due to the interplay of early tuberization, increased chlorophyll content, enhanced rate of photosynthesis, and retaining photosynthetically active leaves longer in response to the treatment. Reduction in tuber number could be linked to the decline in stolon number as result of a decrease in GA activity that may be associated with stolon initiation (Kumar & Wareing, 1972). A strong negative correlation (r = - 0.86*) was observed between tuber fresh mass and number signifying that the substantial increase in individual tuber size was responsible for the yield increment. In agreement with the current finding, PBZ treatment increased tuber yield per plant in the trials of Balamani & Poovaiah (1985) and Simko (1994). However, it is not clear whether the reported yield increments were a consequence of an increase in tuber size or number. On the contrary, Bandara & Tanino (1995) reported that PBZ nearly doubled the number of tubers per plant without affecting the total fresh weight of the tubers. This discrepancy may probably be explained by the cooler growing conditions in their experiment, namely 23 ± 2°C/18 ± 2°C day/night temperature and a 16h day length.

An increase in specific gravity and dry matter content of the tubers in response to PBZ may be attributed to reduced GA activity in the tuber tissue that in turn increased sink strength to attract more assimilates and enhance starch synthesis. Accumulation of GA_3 in tuber tissue reduced sink strength (Booth & Lovell, 1972). Under inductive growing conditions the activities of enzymes involved in potato tuber starch biosynthesis such as ADPG-pyrophosphorylase, starch phosphorylase and starch synthase increase (Visser *et al*., 1994;

Appeldoorn *et al*., 1997). Exogenous application of GA_3 on the growing tubers substantially reduced the activity of ADPG-pyrophosphorylase, while the activity of starch phosphorylase remained more or less constant (Mares *et al*., 1981). Similarly, Booth & Lovell (1972) observed that application of GA_3 to potato shoots reduced export of photosynthates to the tubers, decreased starch accumulation, increased sugar levels and resulted in cessation of tuber growth.

A highly significant positive correlation (r = 0.99**) was observed between specific gravity and percent dry matter, confirming that specific gravity is an excellent indicator of tuber dry matter content. Tsegaw & Zelleke (2002) have also reported a positive correlation between dry matter content and specific gravity of the tubers. Improving the dry matter content of potato tubers with the aid of PBZ treatment may ultimately be useful in the production of tubers having high specific gravity that are suitable for processing.

It has been postulated that PBZ increases tuber crude protein content by counteracting the activity of GA that is known to prevent the induction of tuber protein synthesis. GA_3 treatment inhibits the accumulation of patatin (a glycoprotein associated with tuberization) and other tuber specific proteins (Park, 1990; Vreugdenhil & Sergeeva, 1999). The increase in crude protein content was strongly associated with dry matter content (r = 0.98**) indicating that an increase in tuber dry matter content might have substantially contributed for crude protein gain. Paiva *et al*. (1983) reported that GA regulates starch and patatin accumulations and a close correlation was observed between starch and patatin content.

PBZ treatment significantly extended tuber dormancy. This is in agreement with the results of Harvey *et al*. (1991), Simko (1994), and Bandara & Tanino (1995). This may be associated

with inhibition of GA biosynthesis and prevention of ABA catabolism in response to PBZ treatment (Rademacher 1997). This could result in low GA and high ABA concentrations in the tubers. It has been reported that GA_3 shortens tuber dormancy (Dogonadze *et al*., 2000) while ABA inhibited sprouting by hindering DNA and RNA synthesis (Hemberg, 1970). Prolonging the dormancy period of the tubers with PBZ may be useful for the potato industry to reduce untimely sprouting of potato cultivars with a short dormancy period.

3.6 CONCLUSION

It is concluded that PBZ is an effective plant growth regulator to increase tuber yield and quality under high temperatures and long photoperiods by increasing photosynthetic efficiency and assimilate partitioning to the tubers. The results are of specific importance to increase the productivity of potato in the hot lowland tropics. Using the information as springboard field investigations will be undertaken in the lowland tropics where potato cultivation is restricted due to high temperatures.

CHAPTER 4

PACLOBUTRAZOL INDUCED LEAF, STEM, AND ROOT ANATOMICAL MODIFICATIONS IN POTATO

4.1 ABSTRACT

Plants of potato cultivar BP 1 were treated with 67.5 mg of PBZ per plant as a foliar spray. A month after treatment leaf, stem and root histological observations were made. PBZ treatment resulted in reduced shoot growth, thicker leaves, and increased stem and root diameters. Leaves of treated plants showed increased chlorophyll *a* and *b* contents, had a thicker epicuticular wax layer, and elongated and thicker epidermal, palisade and spongy mesophyll cells. The thickness of the stems was associated with an increase in cortex thickness, enlarged vascular bundles, and larger pith with bigger pith cells. An increase in the width of the cortex and the induction of more secondary xylem vessels in response to PBZ treatment increased the root diameter. PBZ resulted in the accumulation of starch granules in the stem pith cells and cortical cells of the stem and root. Increased leaf thickness, and increased stem and root diameters following application of PBZ has been reported before but the underlying anatomical modifications have not been reported previously.

Keywords: chlorophyll; cortex cell; mesophyll tissue; pith cell; starch granules

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4.2 INTRODUCTION

The regulation of plant growth with synthetic plant growth regulators has become a common agricultural practice. Of the available synthetic plant growth regulators, the triazoles are potent at low concentrations to inhibit shoot growth (Davis *et al.*, 1988). PBZ is a triazole derivative known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway leading to a decrease in endogenous GA levels and ABA catabolism (Rademacher, 1997).

PBZ suppressed growth in a wide range of plant species, and treated plants exhibited a dark green colour, were shorter and more compact in appearance (Terri & Millie, 2000; Sebastian *et al.*, 2002). Plant morphological and anatomical modifications in response to PBZ treatments have been reported in various plant species. Berova & Zlatev (2000) reported a reduced height and an increased stem thickness of tomato in response to PBZ treatment. Treating *Chrysanthemum* plants with PBZ as a soil drench resulted in thicker leaves, reduced stem diameter, and roots with an increased diameter (Burrows *et al*., 1992). Sopher *et al.* (1999) observed thicker and broader maize leaves having more epicuticular wax, enlarged vascular elements, and enlarged epidermal, mesophyll and bundle sheath cells due to PBZ treatment. In wheat, PBZ increased thickness of the leaves by inducing additional layers of palisade mesophyll cells (Gao *et al.*, 1987).

Greenhouse and field experiments on the response of potato grown under non-inductive conditions to PBZ showed that PBZ treatment resulted in compact plants with thicker and dark green leaves. PBZ treatment prevented flower formation. No reports dealing with PBZ

induced anatomical changes in potato are available. The objective of this investigation was to determine the effect of PBZ on leaf, stem, and root anatomy.

4.3 MATERIALS AND METHODS

4.3.1 Plant culture

In a greenhouse experiment on the experimental farm of the University of Pretoria the effect of PBZ on the anatomy of potato leaves, stems and roots was investigated during 2003. Plants of cultivar BP1 were grown in 5-liter plastic containers with a mixture of sand and coconut coir (50:50 by volume) as growing medium. During the growing period diurnal temperatures ranged between 17 and 35 ºC and the average relative humidity was 54%. Plants were fertilized with a standard Hoagland solution and watered regularly to avoid water stress.

4.3.2 Treatments

One month after planting, during early stolon initiation, the plants were treated with PBZ at rates of 0, 45.0, 67.5 and 90.0 mg active ingredient (a.i.) per plant as a foliar spray. (Cultar formulation, 250 g a.i. PBZ per liter, Zeneca Agrochemicals SA (PTY.) LTD., South Africa). The solution was applied as a fine spray using an atomizer and the control plants were treated with distilled water.

4.3.3 Chlorophyll content

Two weeks after treatment, crude leaf chlorophyll extracts were made using 80% acetone. Spectrophotometer (Pharmacia LKB, Ultrospec III) readings were recorded at 663 and 645 nm, and the concentrations of chlorophyll *a* and *b* determined using the specific absorption coefficients recommended by MacKinney (1941).

4.3.4 Morphology and anatomy

Plant height was measured from the base of the stem to the apex. One month after treatment leaf, stem, and root material were collected from the 67.5 mg a.i. PBZ treated plants and control plants. Leaf material was taken from the mid portion of the third youngest fully expanded leaves. Internode samples were taken from the mid portion of the main stem, and the root samples were taken 1 cm below the points of attachment to the stem.

Sections of the leaves, stems, and roots were fixed in formalin/acetic-acid/alcohol (FAA), dehydrated in increasing ethanol concentrations and embedded in paraffin wax (melting point, 58 ºC) after substituting the alcohol with xylene (O'Brien & Mc Cully, 1981). Sections of about 8 µm were made with a rotary microtome and stained in Safranin 0, counter stained in Fast Green, and mounted in Clear Mount (O'Brien & Mc Cully, 1981). Images were made using a Kodak camera (Nikon DXM 1200, Nikon, Japan) fitted on a light microscope (Nikon Opti. Photo, Nikon, Japan). Measurements of leaf anatomical structures were made using image analyser (UTHSCSA Image Tool for Window 3.00).

4.4 RESULTS

PBZ treated leaves were dark green due to high concentrations of chlorophyll *a* (0.82 mg g^{-1} FW) and *b* (0.26 mg g^{-1} FW) (Table 4.1). Leaves of the control treatment contained 0.54 and 0.17 mg g^{-1} FW chlorophyll *a* and *b*, respectively. PBZ treated plants exhibited thicker epicuticular wax layers, larger epidermal cells, a single layer of large and elongated palisade mesophyll cells, and a thicker spongy mesophyll tissue (Figure 4.1B) compared to the control (Figure 4.1A).

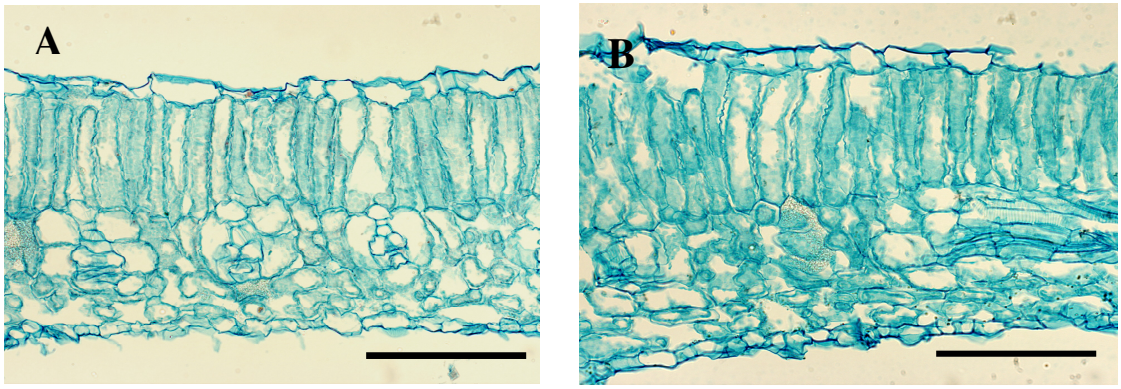

Figure 4.1 Light micrographs of transverse sections of leaves showing enlarged epidermal, palisade mesophyll and spongy mesophyll cells of PBZ treated (B) compared to the control (A). Thicker epicuticular wax deposition can be seen on PBZ treated leaf (B). Scale bar 100 µm

Leaf thickness increased from 215 µm to 268 µm in response to PBZ treatment (Table 4.1). PBZ increased the length and diameter of epidermal cells by about 24 and 14%, respectively over the control (Table 4.1). PBZ treatment increased leaf palisade mesophyll cell length and width (Table 4.1). The mean palisade mesophyll cell length and diameter of the treated leaves were respectively about 116 µm and 21 µm, compared to 88 µm and 15 µm for the untreated leaves. PBZ treatment increased the thickness of spongy mesophyll by about 15% over the control, 96 µm thick.

Table 4.1 Effect of PBZ on leaf, stem and root characteristics. Mean value ± standard deviation

Plant part	Control	PBZ treated	Increase over the control (%)
Leaf			
Chlorophyll a (mg g^{-1} FW)	0.54± 0.05	0.82 ± 0.09	51.8
Chlorophyll b (mg g^{-1} FW)	0.17 ± 0.03	0.26 ± 0.08	52.9
Total thickness (µm)	215.4 ± 5.1	267.8 ± 6.7	24.3
Epidermal cell length (µm)	34.2 ± 13.9	42.3 ± 12.5	23.7
Epidermal cell width (µm)	12.3 ± 3.4	14.0 ± 3.0	13.8
Palisade cell length (µm)	87.6 ± 5.8	116.3 ± 6.4	32.7
Palisade cell width (µm)	14.9 ± 2.6	21.1 ± 3.1	41.6
Spongy mesophyll thickness (µm)	95.6 ± 7.9	110.3 ± 8.0	15.4
Stem			
Stem length (cm)	76.4 ± 1.7	43.5 ± 2.3	-43.1
Stem diameter (mm)	6.6 ± 0.5	10.4 ± 1.2	63.5
Root			
Root diameter (mm)	2.9 ± 0.2	4.4 ± 2.1	51.7

PBZ treatment resulted in shorter and thicker stems compared to the control plants (Table 4.1 and Figure 4.2). The mean plant height was reduced from 76.4 cm to 43.5 cm in response to PBZ treatment while stem diameter was increased by 58% (Table 4.1). This is attributed to the induction of a thicker cortex, well-developed vascular bundles, and a larger pith diameter in response to the treatment (Figure 4.3B). The stem of PBZ treated plants had larger symmetrical pith cells containing numerous starch granules (Figure 4.3D) while the control plants exhibited smaller irregularly shaped pith cells almost devoid of starch granules (Figure 4.3C).

Figure 4.2 Potato plant height reductions in response to PBZ treatment: A = untreated, B = 45 mg a.i. PBZ, C = 67.5 mg a.i. PBZ, and D = 90 mg a.i. PBZ

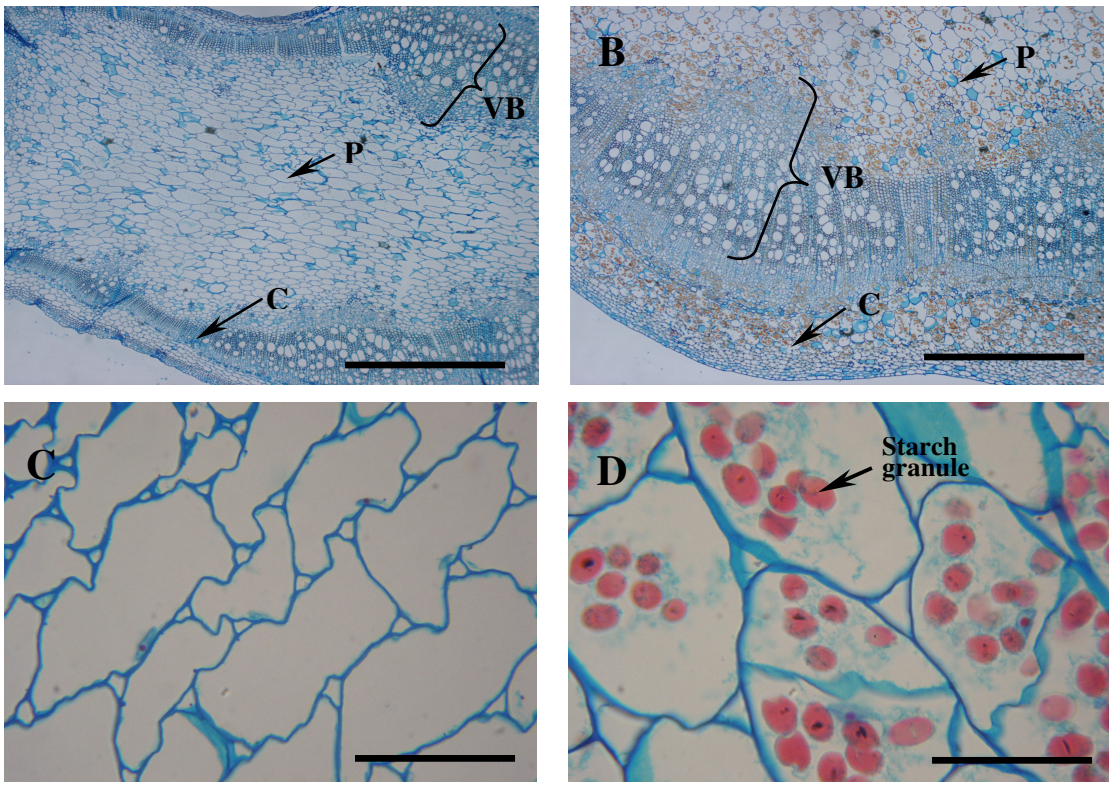

Figure 4.3 Transverse micrographs of sections from the stems of the control and PBZ treated potato plants. The treated stem (B) is characterised by increased cortex thickness (C), well-developed vascular bundles (VB), and wider pith diameter (P) compared to the control (A). Treated plants developed larger, oval shaped pith cells containing starch granules (D) compared to the smaller and irregularly shaped pith cells without starch granules (C). Scale bar 100 µm

The average root diameter of PBZ treated plants was 4.4 mm, 52% thicker than the 2.9 mm of the control (Table 4.1). PBZ increased the width of root cortex and the number of vascular vessels compared to the control (Figure 4.4A and 4.4B). Roots of treated plants developed larger cortical cells containing numerous starch granules (Figure 4.4D) while the untreated plants possessed thin and elongated cortical cells with few starch granules (Figure 4.4C).

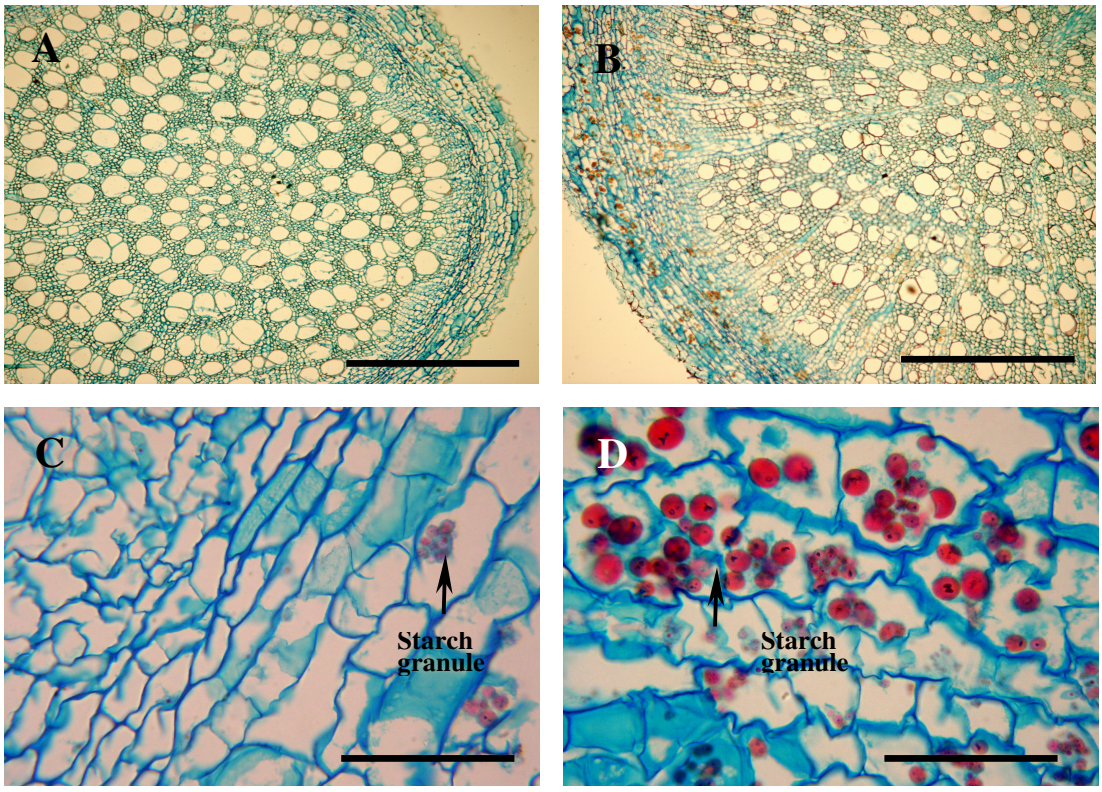

Figure 4.4 Transverse sections of roots of the control and PBZ treated potato plants. Treated plants (B) had larger root diameters due to an increase in the width of the cortex and the induction of more secondary xylem vessels compared to the control (A). Larger root cortical cells of treated plants contained numerous starch granules (D) compared to the smaller cortical cells of the control plants with few starch granules (C). Scale bar 100 µm

4.5 DISCUSSION

PBZ treated potato plants exhibited a dark green colour due to high chlorophyll *a* and *b* contents. The increase in chlorophyll content may be attributed to enhanced chlorophyll synthesis and/or more densely packed chloroplasts per unit leaf area. Sebastian *et al.* (2002) reported enhanced chlorophyll synthesis in *Dianthus caryophyllus*, and Khalil (1995) observed more densely packed chloroplasts per unit leaf area in response to PBZ treatment. Increased chlorophyll content in potato due to PBZ treatment was observed by Balamani & Poovaiah (1985) and Bandara & Tanino (1995). The higher chlorophyll content of treated potato leaves may be related to the influence of PBZ on endogenous cytokinin levels. It has been proposed that PBZ stimulates cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher *et al.*, 1982). GA biosynthesis inhibitors increased cytokinin content in soybean (Grossman, 1992) and *Dianthus caryophyllus* (Sebastian *et al.*, 2002).

The observed higher epicuticular wax deposition on treated leaves may be related to the increase in endogenous ABA levels in response to PBZ treatment (Rademacher, 1997). An increase in ABA stimulates the synthesis of lipid transfer proteins in barley that play an important role in the formation of epicuticular waxes, a process that affects the water relation of the leaves (Hollenbach *et al.*, 1997). PBZ treatments caused an increase of 10% in total wax load and change the proportion of certain wax constituents in potted rose cultivars within 11 days of application (Jenks *et al*., 2001). The development of a thicker epicuticular wax layer provides better protection against some plant pathogens and minor mechanical damage (Kolattukudy, 1987).

The observed increase in leaf thickness is attributed to an increase in epidermal cell diameter, palisade cell length and spongy mesophyll depth. Burrows *et al.* (1992) reported that increased *Chrysanthemum* leaf thickness in response to PBZ treatment was due to thicker spongy mesophyll, and the induction of additional layers of palisade parenchyma, although individual cells were shorter, of small diameter and more tightly packed. In maize PBZ treated leaves showed more epicuticular wax deposition and were thicker and broader owing to enlarged vascular elements, epidermal, mesophyll, and bundle sheath cells (Sopher *et al.*, 1999). Hawkins *et al.* (1985) reported a 15-24% increase in soybean leaf thickness due to the elongation of the palisade cells without affecting the number of palisade rows and spongy parenchyma thickness. Dalziel & Lawrence (1984) reported that PBZ induced a 100% increase in sugar beet leaf thickness due to a three to four fold increase in palisade cell length, without affecting the number of rows.

PBZ treated potato plants were shorter and had thicker stems than the control. Reduced internode length caused height reduction. Davis & Curry (1991) reported that shoot growth reduction in response to PBZ treatment occurs primarily due to a decrease in internode length, and the effective dose varies with species and cultivar. This response may probably be explained by the reduction in the endogenous GA level. GA enhances internode elongation of intact stems (Salisbury & Ross, 1992). Liu & Loy (1976) showed that GA promote cell division by stimulating cells in the G_1 phase to enter the S phase and by shortening the duration of S phase. They concluded that increased cell numbers lead to more rapid stem growth. Similar reductions in shoot growth were reported in Scaevola (Terri & Millie, 2000) and *Dianthus caryophyllus* (Sebastian *et al.*, 2002) in response to PBZ treatment. More recently, Suzuki *et al.* (2004) reported that the presence of PBZ in the medium strongly inhibited etiolated and non-etiolated longitudinal shoot growth of *Catasetum fimbriatum.*

PBZ treatment increased cortex thickness, size of the vascular bundles, and pith diameter and resulted in thicker stems. This modification may be attributed to radial expansion of cells due to reduced endogenous GA activities in response to the treatment. Wenzel *et al.* (2000) reported that GA limits the extent of radial expansion of plant organs. In dicot stems, cell shape alterations are apparently caused by a more longitudinal orientation of cellulose microfibrils being deposited in the cell walls, preventing expansion parallel to the these microfibrils but allowing expansion perpendicular to them (Eisinger, 1983). The non-uniform distribution and arrangement of the vascular elements in the potato stems resulted in irregularity in the shape of the stems. Various authors reported different results in various plant species with respect to PBZ induced stem anatomy modifications. PBZ reduced both cell number and length in safflower stem (Potter *et al.*, 1993). Burrows *et al*. (1992) reported that PBZ treatment brought about a 50% reduction in *Chrysanthemum* stem diameter because of an enhanced development of secondary xylem and a marked reduction in the number of sclerenchyma bundle caps. In peach shoots, PBZ reduced the proportion of xylem and increased that of phloem and cortex, and increased xylem density (Aguirre & Blanco, 1992). In an investigation on poinsettia, McDaniel *et al.* (1990) found that PBZ application suppressed cell wall thickening in the phloem fiber caps, decreased the width of xylem ring, and disfavoured the differentiation of interfasicular supporting tissues.

It was observed that untreated plants had more, thinner and longer roots compared to the treated plants. PBZ increased root diameter by increasing the width of the cortex and by favouring the formation of more secondary xylem vessels. Depending on the plant species and the concentration, PBZ either stimulated or inhibited root growth. PBZ caused thickening of maize roots and increased their starch content (Baluska *et al.*, 1993). Treating primary roots of pea inhibited root extension but promoted radial cell expansion (Wang & Lin, 1992). Increased root

diameter has been correlated with larger cortical parenchyma cells in soybean and maize (Barnes *et al.*, 1989). Increasing root diameter in *Chrysanthemum* was due to an increase number of rows and diameter of cortical cells (Burrows *et al.*, 1992). A stimulatory effect of PBZ on root growth has also reported in English ivy (Geneve, 1990) and mung bean (Porlingis & Koukourikou-Petridou, 1996).

PBZ increased the accumulation of starch granules in the pith cells of the stem, and in the cortical cells of the stems and roots. It is postulated that the increase in the number of starch granules may be attributed to PBZ stimulated reduction in the GA activity. Under favourable conditions for tuberization (GA content below threshold level), the activities of enzymes involved in potato tuber starch biosynthesis such as ADPG-pyrophosphorylase, starch phosphorylase and starch synthase increase (Visser *et al.*, 1994; Appeldoorn *et al.*, 1997). Mares *et al.* (1981) observed that exogenous application of GA_3 on growing tubers substantially reduced the activity of ADPG-pyrophosphorylase, while the activity of starch phosphorylase remained more or less constant. Booth & Lovell (1972) reported that application of GA_3 to potato shoots reduced starch accumulation in the tubers. PBZ treatment increased root starch content in maize plants (Baluska *et al.,* 1993). PBZ treatment increased starch accumulation in the leaves, stems, crowns and roots of rice seedling while GA_3 treatment decreased starch accumulation in the leaves and crowns of the seedlings (Yim *et al.*, 1997).

4.6 CONCLUSION

PBZ modified the morphology of the potato plant in such a way that treated plants appeared to be dark green, and short and compact. Darkness of the leaves was due to an increase in chlorophyll *a* and *b* concentrations. The induction of elongated and thicker epidermal, palisade and spongy mesophyll cells in response of PBZ treatment increased leaf thickness. The thickness of the stems was correlated with an increase in cortex thickness, enlarged vascular bundles, and larger pith with bigger pith cells. An increase in the thickness of cortex and the induction of more secondary xylem vessels increased the root diameter. PBZ enhanced starch synthesis in the pith cells of the stem and cortical cells of the stems and roots. This study confirms that PBZ treatment can induce morphological and anatomical modifications in potato similar to those reported in a wide range of plant species.

CHAPTER 5

RESPONSE OF POTATO GROWN IN A HOT TROPICAL LOWLAND TO PACLOBUTRAZOL. I: SHOOT ATTRIBUTES, PRODUCTION AND ALLOCATION OF ASSIMILATES

5.1 ABSTRACT

The growth response of potato to PBZ under the hot tropical condition of eastern Ethiopia was investigated in two field experiments. A month after planting PBZ was applied as a foliar spray or soil drench at rates of 0, 2, 3, and 4 kg a. i. PBZ per ha. Regardless of the method of application, PBZ increased chlorophyll *a* and *b* content and net rate of photosynthesis, but reduced shoot growth, plant height, stomatal conductance and the rate of transpiration. PBZ delayed the onset of leaf senescence and increased the partitioning of assimilates to the tubers while reducing assimilate supply to the leaves, stems, roots and stolons. PBZ improved the productivity of potatoes grown in the hot tropical lowlands by reducing shoot growth, increasing leaf chlorophyll content, enhancing the rate of photosynthesis, improving water use efficiency, and increased partitioning of dry matter to the tubers.

Keywords: Assimilation; chlorophyll content; photosynthesis; senescence; stomatal conductance; transpiration

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5.2 INTRODUCTION

Lowland tropical regions are characterized by high temperatures that limit successful potato cultivation (Midmore, 1984). In Ethiopia about 35 % of the available agricultural land is situated in semi-arid regions of the country, where high temperatures throughout the year limit potato production.

Leach *et al.* (1982) developed a detailed carbon budget for potato indicating that plant growth rate is strongly related to net photosynthesis and dark respiration. In the tropics, of the gross carbon fixed up to 50% may be utilized for respiration (Burton, 1972). Respiration increases with temperature and it is estimated to roughly double for each 10 ºC increase between 10 ºC and 35 ºC (Sale, 1973). Above 30 ºC the rate of net photosynthesis declines rapidly (Leach *et al.,* 1982; Thornton *et al.* 1996). Hence, reduced assimilate production due to decreased photosynthesis and increased respiration are important factors limiting potato productivity in hot tropical lowlands.

The most noticeable morphological features of potatoes grown under high temperatures are taller plants with longer internodes, increased leaf and stem growth, decreased leaf: stem ratio, and shorter and narrower leaves with smaller leaflets (Menzel, 1985; Manrique, 1989; Struik *et al.,* 1989). Although there are genetic differences (Manrique, 1989; Hammes & De Jager, 1990), high temperatures decrease the partitioning of assimilate to the tubers and increase partitioning to other parts of the plant (Wolf *et al.,* 1990; Vandam *et al.,* 1996). Under long photoperiods, high temperatures may shift partitioning of assimilates to the shoots thereby delaying leaf senescence (Struik *et al.,* 1989); but under short photoperiods, high temperatures favour rapid growth and development and shortens the growing period (Vander

Zaag *et al.,* 1990). Higher temperatures favour the production of high levels of GA-like compounds in potato plants (Menzel, 1983).

PBZ is a triazole plant growth regulator known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway (Rademacher, 1997). Interference with the different isoforms of this enzyme could lead to inhibition of GA biosynthesis and prevention of abscisic acid (ABA) catabolism. In addition, PBZ induces various plant responses such as shoot growth reduction (Terri & Millie, 2000; Sebastian *et al.,* 2002), enhanced chlorophyll synthesis (Sebastian *et al.,* 2002), delayed leaf senescence (Davis & Curry, 1991), improved water use by reducing the rate of transpiration (Ritchie *et al.,* 1991; Sankhla *et al.,* 1992; Eliasson *et al.,* 1994) and increased assimilate partitioning to the underground parts (Balamani & Poovaiah, 1985; Davis & Curry, 1991; Bandara & Tanino, 1995).

Greenhouse experiments on the effect of PBZ on potato growth suggested that it enhances the productivity under non-inductive conditions (Chapter 3). It is proposed that PBZ reduces GA biosynthesis in potatoes, and should improve productivity in the lowland tropics and improves productivity. This paper reports the effect of foliar and root applied PBZ on shoot growth, chlorophyll content, stomatal conductance, rate of transpiration, photosynthetic efficiency as well as biomass production and partitioning in potato grown under hot tropical conditions in the lowland of eastern Ethiopia. As a follow up from the same experiments, growth analyses and tuber attributes are presented in Chapter 6 and Chapter 7, respectively.

5.3 MATERIALS AND METHODS

5.3.1 Site description

Two similar field experiments were conducted under irrigation from January to July 2003 at Tony Farm, research farm of Alemaya University, Ethiopia. The site is located at 41° 50.4' E longitude, 09° 36' N latitude, at an altitude of 1176 m.a.s.l. in the semi-arid tropical belt of eastern Ethiopia. During the growing period the total precipitation was 230 mm and the mean monthly minimum and maximum temperatures were 18 ºC (ranging from 15.4 to 21.3 ºC) and 31 ºC (ranging from 28.0 to 34.4 ºC), respectively. The mean relative humidity was 50%, varying from 20 to 81%. The soil was a well-drained deep clay loam with 2.36% organic matter, 1.36% organic carbon, 0.12% total nitrogen, 14.15 ppm phosphorus, 1.08 meq100 g^{-1} exchangeable potassium, 0.533 mMhoscm^{-1} electric conductivity and a pH of 8.6.

5.3.2 Plant culture

Treatments were laid down as two-factor (rate and method of application) factorial experiments arranged in randomised complete block designs with three replications. In each plot (5.25 m x 2.1 m) forty-nine medium sized, well sprouted tubers of cultivar 'Zemen' were planted at a spacing of 75 x 30 cm. Phosphorus was applied as diammonium phosphate at planting time at a rate of 150 kg P ha^{-1} and nitrogen was side dressed after full plant emergence at a rate of 100 kg N ha^{-1} in the form of urea. The plots were furrow irrigated regularly to maintain adequate moisture in the soil. Standard cultural practices for regional potato production were applied (Teriessa, 1997) and no pests or diseases of importance were observed.

5.3.3 Treatments

Thirty days after planting (early stolon initiation) the plants were treated with PBZ at rates of 0, 2, 3, and 4 kg active ingredient (a.i.) PBZ ha^{-1} as a foliar application or soil drench using the Cultar formulation (250 g a.i. PBZ per liter, Zeneca Agrochemicals SA (PTY.) LTD., South Africa). To prepare the aqueous solutions PBZ was diluted in distilled water (250 ml $plot^{-1}$). For the foliar treatment, the solution was applied to each plant as a fine foliar spray using an atomizer. While applying the foliar treatment, the soil was covered with a plastic sheet to avoid PBZ seepage to the ground. The drench solution was applied to the soil in a ring around the base of each plant. The control plants were treated with distilled water at equivalent volumes.

5.3.4 Data recorded

Two weeks after treatment application stomatal conductance, rate of transpiration and photosynthesis were measured using a portable LCA4 photosynthesis system (ADC Bio Scientific Ltd., UK) and leaf chlorophyll content was determined. The measurements were made on three randomly selected plants using the terminal leaflets of the 2^{nd}, 3^{rd} and 4^{th}, fully expanded younger leaves. To determine the concentrations of chlorophyll *a* and *b*, spectrophotometer (Pharmacia LKB, Ultrospec III) readings of the density of 80% acetone chlorophyll extracts were taken at 663 and 645 nm, and their respective values were assessed using the specific absorption coefficients given by MacKinney (1941).

Directly after treatment application and two, four, six, and eight weeks after treatment, three randomly selected plants were harvested from each plot. Samples were separated into leaves, stems, tubers, and roots and stolons. Leaf area of photosynthetically active green leaves was

measured with a portable CI-202 leaf area meter (CID Inc., Vancouver, Washington state, USA). Plant tissue were oven dried at 72 °C to a constant mass. Dry matter partitioning was determined from the dry mass of individual plant components as a percentage of the total plant dry mass. Plant height was measured from the base of the stem to shoot apex. Days to physiological plant maturity were recorded when 50% of the leaves turned yellow.

5.3.5 Statistical analysis

Analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C, 1991). Means were compared using least significant differences (LSD) test at 1% probability level. Correlations between parameters were computed when applicable. Combined analysis of variance of the two experiments revealed that there was no significant treatment by experiment interaction. Hence, pooled data are presented for discussion.

5.4 RESULTS

There were no significant differences between the foliar spray and soil application with respect to chlorophyll content, stomatal conductance, rate of transpiration, and plant height. Means pooled over methods of application showed that PBZ treatments reduced total leaf area (Figure 5.1). PBZ treatment resulted in a significant height reduction and application of 3 or 4 kg a.i. PBZ ha^{-1} resulted in a mean reduction of 63% in stem length (Table 5.1).

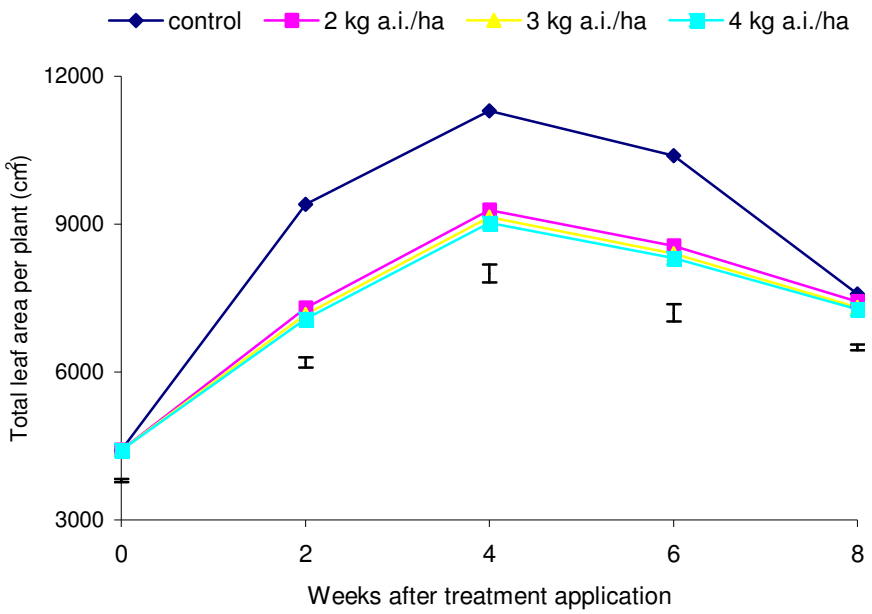

Figure 5.1. Total leaf area of potato plants grown under hot tropical lowland conditions as influenced by rates of PBZ application. The vertical bars represent least significant differences at P < 0.01

The concentrations of chlorophyll *a* and *b* in leaf tissue were significantly increased with PBZ treatments (Table 5.1). Compared to the control, application of 3 or 4 kg a.i. PBZ ha^{-1} increased the chlorophyll *a* content of leaf tissue by an average of 65%. In the same manner, regardless of the concentrations, PBZ treatment increased the chlorophyll *b* content by an average of 55% compared to the control. Total leaf area negatively correlated with chlorophyll *a* (r = - 0.93**) and chlorophyll *b* (r = - 0.97**) content.

Irrespective of the rate of application, PBZ treatment greatly reduced leaf stomatal conductance and rate of transpiration (Table 5.1). The lowest stomatal conductance (0.16 mol m^{-2} s^{-1}) and rate of transpiration (3.78 mol m^{-2} s^{-1}) values were recorded for plants that received 4 kg a.i. PBZ ha^{-1}. In contrast, PBZ treatment enhanced the rate of leaf net photosynthesis, with the highest value observed in plants treated with 3 or 4 kg a.i. PBZ ha^{-1}.

Table 5.1 Chlorophyll *a* and chlorophyll *b* concentrations, stomatal conductance (Gs), rate of transpiration (E), net photosynthesis (Pn) of leaf tissue, and potato plant height as influenced by rates of PBZ application

Rate (a.i. kg ha^{-1})	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Gs (mol m^{-2} s^{-1})	E (mol m^{-2} s^{-1})	Pn (µmol m^{-2} s^{-1})	Plant height (cm)
0 (control)	0.50c	0.15b	0.25a	5.00a	6.47b	77.92a
2	0.68b	0.22a	0.19b	3.97b	7.34ab	33.02b
3	0.81a	0.23a	0.18b	4.08b	8.40a	30.03bc
4	0.84a	0.25a	0.16b	3.78b	8.21a	27.63c
SEM	0.03	0.01	0.02	0.26	0.36	0.81

SEM: Standard error of the mean.

Means within the same column sharing the same letters are not significantly different ($P < 0.01$).

A significant interaction between application method and PBZ application rate was observed for days to physiological maturity (Table 5.2). Compared to the control, regardless of the concentrations, foliar spray of PBZ delayed the onset of senescence by an average of 17 days, while applying 3 or 4 kg a.i. PBZ ha^{-1} as a soil drench delayed the maturity by about 15 days.

Table 5.2 Days to physiological maturity for potato plants grown in a hot tropical lowland as influenced by PBZ application method and rate

Application method	PBZ rate (a.i. kg ha^{-1})			
	0 (control)	2	3	4
Foliar spray	83.00e	100.83a	100.83a	100.00ab
Soil drench	83.17e	97.33d	98.00cd	99.17bc
SEM	0.38			

SEM: standard error of the mean.

Means within columns and rows sharing the same letters are not significantly different ($P < 0.01$).

PBZ significantly affected total dry matter production and assimilate allocation to the different plant parts (Table 5.3). At all harvesting stages PBZ treatment greatly reduced the dry mass of the leaves, stems, and roots and stolons, and increased the tubers. At the first harvest, tubers were present on PBZ treated plants, while the control had not yet initiated tubers. At the second and

third harvests, tubers represented about 31 and 36% of the total dry mass of PBZ treated plants, and only 14 and 22% in the case of untreated plants. Correspondingly, at the fourth harvest, the plants treated with 3 or 4 kg a.i. PBZ ha^{-1} had partitioned about 40% of the assimilates to the tubers, compared to 26% in the control. Foliar application of PBZ increased total biomass production more than the soil drench during the third and fourth harvesting periods.

Table 5.3 Total dry matter production (g) and distribution (%) amongst different parts of potato plants grown under a hot tropical condition, as influenced by rate and method of PBZ application

Treatment	Total (g)	Leaves (%)	Stems (%)	Roots & stolons (%)	Tubers (%)	Total (g)	Leaves (%)	Stems (%)	Roots & stolons (%)	Tubers (%)
	---------- Harvest I ----------					---------- Harvest II ----------				
Foliar spray	48.9a	43.3a	27.7a	10.1a	18.8a	92.5a	38.3a	24.5a	10.5a	26.7a
Soil drench	46.7a	44.1a	27.2a	10.1a	18.6a	89.0a	39.9a	23.6a	10.2a	27.2a
SEM	0.50	0.52	0.44	0.20	0.31	0.70	0.47	0.31	0.20	0.33
0 (control)	51.3a	53.6a	34.5a	11.9a	0.0c	99.0a	43.5a	29.2a	13.5a	13.7b
2	50.5a	42.4b	25.6b	9.3b	22.7b	90.3b	37.2b	22.7b	9.8b	30.2a
3	46.3b	38.7c	25.1b	9.3b	26.5a	88.6bc	36.7b	22.0b	9.3bc	31.9a
4	43.2c	40.2bc	25.0b	9.4b	25.4a	85.0c	37.0b	22.3b	8.7c	32.a
SEM	0.71	0.75	0.62	0.28	0.44	0.99	0.66	0.44	0.28	0.46
	---------- Harvest III----------					---------- Harvest IV ----------				
Foliar spray	129.7a	34.1b	23.5a	9.6b	32.4a	151.9a	32.4a	23.2a	9.0b	35.1a
Soil drench	124.6b	35.5a	23.0a	9.0a	32.5a	146.9b	33.1b	22.5a	8.4a	36.0b
SEM	0.78	0.26	0.33	0.13	0.28	0.82	0.21	0.32	0.13	0.18
0 (control)	138.0a	39.8a	26.3a	11.9a	22.0b	162.2a	37.3a	25.8a	11.1a	25.8c
2	125.1b	33.3b	22.4a	8.8b	35.5a	146.3b	31.3b	22.1b	8.2b	38.4b
3	124.4b	33.4b	22.3b	8.4b	35.9a	146.3b	31.2b	21.9b	7.7b	39.0ab
4	121.2b	33.4b	22.0b	8.1b	36.5a	142.6b	31.2b	21.6b	7.6b	39.6a
SEM	1.10	0.37	0.47	0.19	0.40	1.16	0.30	0.46	0.18	0.35

Harvest I, II, III and IV done two, four, six and eight weeks after treatment application.

SEM: standard error of the mean.

Means of the same main effect within the same column sharing the same letters are not significantly different ($P < 0.01$).

5.5 DISCUSSION

PBZ is a potent synthetic plant growth regulator and at low concentrations induces physiological, anatomical and morphological changes in plants. The most striking growth response of potato to PBZ treatment was reduced shoot growth. Treated plants were short and compact due to the reduction in total leaf area and stem elongation. Davis & Curry (1991) reported that depending on the species and cultivar, PBZ reduced shoot growth mainly by reducing internode length. It is postulated that reduced GA synthesis in response to PBZ treatment may have resulted in a reduction in cell proliferation leading to a reduction in stem elongation and leaf expansion. In support of this postulation, Haughan *et al.* (1989) reported that the 2R configuration of PBZ greatly retarded cell proliferation in celery. PBZ effectively suppressed growth in a wide range of plant species and the treated plants tended to be darker, and more compact in appearance (Kamoutsis *et al.*, 1999; Terri & Millie, 2000; Sebastian *et al.,* 2002).

The foliage of PBZ treated potato plants typically exhibited a dark green colour compared to the control. This may be due to an increase in chlorophyll content of the leaves either as the result of enhanced chlorophyll synthesis and/or the presence of more chloroplasts per unit leaf area of treated leaves. The observed negative correlation between total leaf area and chlorophyll content indicate that the reduction in total leaf area in response to PBZ treatment contributed to the increased chlorophyll *a* and *b* content. Balamani & Poovaiah (1985) and Bandara & Tanino (1995) also reported an increased chlorophyll concentration in potato leaves in response to PBZ treatment. Increased chlorophyll synthesis due to PBZ treatment was reported in *Dianthus caryophyllus* (Sebastian *et al*., 2002). Investigations undertaken by Khalil (1995) on cereals showed the existence of more densely packed chloroplast per unit leaf area in response to PBZ treatment.

The higher chlorophyll content and delayed senescence in the treated potato leaves may be related to the influence of PBZ on endogenous cytokinins. It has been proposed that PBZ stimulates cytokinin synthesis which increases chloroplast differentiation and chlorophyll biosynthesis, and prevents chlorophyll degradation (Fletcher *et al.,* 1982). Investigations on rice (Izumi *et al.,* 1988), soybean (Grossman, 1992) and *Dianthus caryophyllus* (Sebastian *et al.,* 2002) showed that exogenous application of GA biosynthesis inhibitors increased cytokinin content of plant tissues. The onset of senescence was considerably delayed with the aid of triazoles in several plant species and treated leaves were retained longer than the untreated leaves (Davis & Curry, 1991; Binns, 1994).

PBZ treatments significantly reduced the rate of transpiration in potato leaves. This could be due to the partial closure of stomata in response to PBZ treatment as shown in the concomitant reduction in stomatal conductance. It is postulated that the reduction in stomatal conductance in response to PBZ treatment may have been mediated through its effect on the endogenous ABA content (Rademacher, 1997), as ABA is involved in regulating the opening and closing of stomata (Salisbury & Ross, 1992). Asare-Boamah *et al*. (1986) observed a reduction in the rate of transpiration, increased diffusive resistance and a transient rise in ABA levels in response to triazole treatment. This response may improve the drought tolerance of potato plants. PBZ treatment has been shown to reduce water loss and improve water use efficiency in grapevine, *Chrysanthemum*, and beetroot (Ritchie *et al.,* 1991; Smith *et al.,* 1992; Roberts & Mathews, 1995).

In contrast to its effect on stomatal conductance, PBZ increased photosynthetic efficiency. This response could be linked to the increase in chlorophyll concentration and earlier tuberization. Previous studies on carbon fixation and allocation in various crops showed that the source: sink

balance influence the rate of photosynthesis in such a way that an increased sink demand increased the rate of photosynthesis and a decreased sink demand decreased photosynthesis (Geiger, 1976; Hall & Milthorpe, 1978; Peet & Kramer, 1980). A similar interaction has been observed in the potato. Nosberger & Humphries (1965) reported that removal of growing tubers reduced the rate of net photosynthesis, while tuber initiation increased the rate of net photosynthesis (Moorby, 1968; Dwelle *et al.,* 1981a). Similarly, Basu *et al*. (1999), from a tuber detachment experiment reported that within 6 hours of tuber removal, light saturated rates of net photosynthesis declined from 22 μmol m^{-2} s^{-1} to a value close to zero. Increased net photosynthesis in response to PBZ treatment has been reported in soybean (Sankhla *et al.,* 1985) and rape (Zhou & Xi, 1993). Reduced stomatal conductance did not lead to reduced net photosynthesis. This may be related to PBZ induced modification of the photosynthetic tissue (mesophyll) that may have allowed better diffusion of CO_2 to carboxylation sites. De Greef *et al.* (1979) reported that rate of photosynthesis increased as the mean cell size increased, because bigger mesophyll cells have larger surface to volume ratio. Microscopic observation showed that PBZ increased the size of epidermal, palisade and spongy mesophyll cells of potato leaves (Chapter 4).

PBZ affected the overall pattern of carbon fixation and assimilate partitioning to the different potato organs. Tubers were the dominant sinks that attracted the highest proportion of dry matter relative to the leaves, stems, roots and stolons. This dominance may be linked to low GA concentrations in tubers due to PBZ treatment, thus increasing tuber sink strength. This postulations is based on results by Menzel (1980) and Mares *et al.* (1981) who reported that exogenous GA_3 application inhibited tuber formation; decreased tuber sink strength and encouraged shoot and stolon growth. High temperatures decrease tuber growth rate, reduce the partitioning of assimilates to the tubers and increase assimilation to other parts of the plant

probably associated with high GA levels (Menzel, 1980; Struik *et al.,* 1989; Vandam *et al.,* 1996).

5.6 CONCLUSION

The field trials indicated that PBZ treatment increased leaf chlorophyll content and enhanced the rate of net photosynthesis. PBZ potentially reduce water demand reducing leaf area, and stomatal conductance and the rate of leaf transpiration. PBZ also reduced shoot growth and increased partitioning of assimilates to the tubers. There was no difference between foliar spray and soil drench for most of the parameters considered.

CHAPTER 6

RESPONSE OF POTATO GROWN IN A HOT TROPICAL LOWLAND TO PACLOBUTRAZOL. II: GROWTH ANALYSES

6.1 ABSTRACT

Two similar field trials were carried out during 2003 in a hot tropical region of eastern Ethiopia to investigate the effect of leaf and soil applied PBZ on the growth, dry matter production and partitioning in potato. A month after planting PBZ was applied as a foliar spray or soil drench at rates of 0, 2, 3, and 4 kg a. i. PBZ per ha. Plants were sampled directly after treatment application and subsequently two, four, six and eight weeks later. The data was analysed using standard growth analyses techniques. None of the growth parameters studied was affected by the method of PBZ application. PBZ decreased leaf area index, and crop growth rate, and increased specific leaf weight, tuber growth rate, net assimilation rate, and partitioning coefficient of potato. Although PBZ decreased crop growth rate, it improved tuber yield by partitioning more assimilates to the tubers. PBZ improved the productivity of potato under tropical conditions by improving assimilate allocation to the tubers.

Keywords: Assimilation, growth analysis; high temperature; potato; tropical lowlands;

Publication based on this study:

Tekalign, T. and Hammes, P. S. 2005. Growth and biomass production in potato grown in the hot tropics as influenced by paclobutrazol. ***Plant Growth Regul***. (accepted for publication on November 18, 2004)

6.2 INTRODUCTION

Potato prefers cool weather and temperatures between 16 - 25 ^{o}C favour foliage growth, net photosynthesis, and tuberization (Levy, 1992). Although potato is a remarkably adaptable crop, its expansion has been restricted by high temperatures in some regions of the world (Levy, 1986). For instance, in Ethiopia about 35 % of the available agricultural land is situated in semi-arid regions of the country, where potato cultivation has not been practiced due to unfavourably high temperatures throughout the year. High temperatures in the tropics cause yield reduction and are considered the major constraints for potato production. Yield reduction is due partly to reduced assimilate production, delayed tuber initiation, and reduced assimilate partitioning to the tubers (Ewing, 1981; Menzel, 1980; Struik *et al.,* 1989; Vandam *et al.,* 1996)

The total dry matter yield of crops depends on the size of leaf canopy, the rate at which the leaf functions (efficiency), and the length of time the canopy persists (duration). A study of dry matter production and distribution to the various plant parts in the course of development is important for the evaluation of the growth rate, productivity and the yield level of potato.

Growth analysis has widely been used to analyse yield-influencing factors, explains observed differences in productivity and characterize plant development (Gardner *et al*., 1985). The commonly used growth analysis parameters are leaf area index, relative growth rate, net assimilation rate, and crop growth rate (Gardner *at al.,* 1985). Leaf area index is the ratio of leaf surface to the ground area occupied by the crop. Relative growth rate expresses dry matter weight increase in a time in relation to initial weight. Net assimilation rate is net gain of assimilates per unit leaf area and time. Gain in weight of community of plants on a unit of land in a unit of time reflects crop growth rate.

The production of assimilates by the leaves (source) and the extent to which it can be accumulated in the sink organs, determines crop yield (Hahn, 1977). Assimilate partitioning to the different sinks may be controlled by environmentally regulated hormonal balances (Almekinders & Struik, 1996). Yim *et al.* (1997) suggested the involvement of GA in regulating the pattern of assimilate partitioning in such a way that high GA level leads to a higher carbohydrate allocation to the shoots, where as low GA level resulted in more dry matter allocation to the roots.

PBZ is a triazole compound and interferes with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation path to block GA biosynthesis (Rademacher, 1997). It is proposed that PBZ treatment modifies the growth of potato under high temperature regimes by affecting growth parameters such as leaf area, specific leaf weight, net assimilation rate, and crop growth rate and tuber growth rate. This chapter presents analyses of the growth response of potato to paclobutrazol in a hot tropical region in eastern Ethiopia.

6.3 MATERIALS AND METHODS

6.3.1 Site description

Details of the site are presented in Chapter 5.

6.3.2 Plant culture

Cultural methods are described in Chapter 5.

6.3.3 Treatments

The treatments that were applied are presented in Chapter 5.

6.3.4 Data recorded

Directly after treatment application, and two, four, six, and eight weeks afterwards, three randomly selected plants were harvested from each plot. The samples were separated into leaves, stems, tubers, and roots and stolons. Green leaf area was measured with a portable CI-202 leaf area meter (CID Inc., Vancouver, Washington State, USA). Plant tissues were oven dried at 72 °C to a constant mass.

Growth analyses were conducted by computing the following standard formulae:

$LAI = [(L_{A2} + L_{A1})/2] * (1/G_A)$ (Gardner *et al.,* 1985)

$SLW = (L_{W2}/L_{A2} + L_{W1}/L_{A1})/2$ (Gardner *et al.,* 1985)

$CGR = 1/G_A * (W_2 - W_1) / (t_2 - t_1)$ (Gardner *et al.,* 1985)

$TGR = 1/G_A * (T_2 - T_1) / (t_2 - t_1)$ (Manrique, 1989)

$NAR = [(W_2 - W_1) / (t_2 - t_1)] * (\ln L_{A2} - \ln L_{A1})/ (L_{A2} - L_{A1})]$ (Gardner *et al.,* 1985)

$PC = TGR / CGR$ (Duncan *et al.,* 1978)

Where:

LAI is leaf area index; L_{A2} and L_{A1} are leaf area at time 2 (t_2) and time 1 (t_1), respectively; G_A ground area covered by the crop; SLW is specific leaf weight expressed in g cm^{-2}, L_{W2} and L_{W1} are leaf dry mass at time 2 (t_2) and time 1 (t_1), respectively; CGR is crop growth rate expressed in g m^{-2} day^{-1}, W_2 and W_1 are total crop dry mass (g) at t_2 and t_1; TGR is tuber growth rate expressed in g m^{-2} day^{-1}; T_2 and T_1 are tuber dry mass (g) at t_2 and t_1; NAR is net assimilation rate expressed in g m^{-2} day^{-1}; PC is partitioning coefficient.

6.3.5 Statistical analysis

The analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C 1991). Combined analysis of variance showed no significant treatments by experiment interactions. Hence, for all of the parameters considered, the data of the two experiments were combined. Means were compared using the least significant differences (LSD) test at 5% probability level. Trends in different growth parameters were analysed by a linear regression, using Microsoft Excel 2000.

6.4 RESULTS

Leaf area index, specific leaf weight, relative growth rate, crop growth rate, tuber growth rate, net assimilation rate as well as partitioning coefficient were not affected by the method of PBZ application and consequently only the graphs of main effects of the treatment rates are presented.

PBZ significantly decreased the leaf area index compared to the control (Figure 6.1). The peak leaf area index for both treated (LAI = 3.9) and the control (LAI = 4.8) plants were attained 6

weeks after treatment application, about 60 days after planting. Irrespective of the concentration, PBZ treatment reduced leaf area indices by about 16, 21 and 19 % during the 2nd, 4th and 6th week after treatment application.

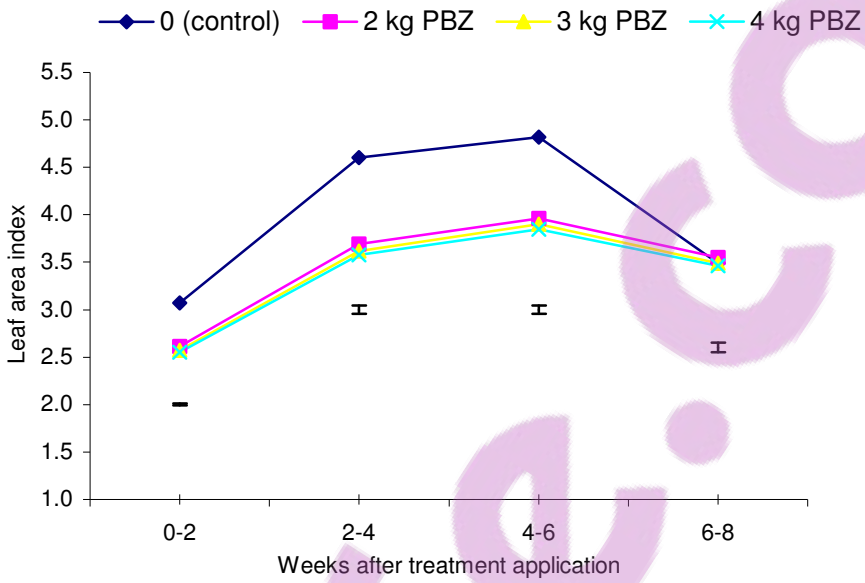

Figure 6.1. Leaf area index of potato as affected by rates of PBZ. The vertical bars represent least significant differences at P < 0.05

At all harvesting stages except for the first, PBZ increased the specific leaf weight (Figure 6.2). The highest specific leaf weight value for the control (4.1 mg cm^{-2}) as well as PBZ treated plants (4.3 mg cm^{-2}) were attained 4-6 weeks after treatment. The specific leaf weight increased sharply up to 6 weeks after treatment, and tended to decline slightly by week eight.

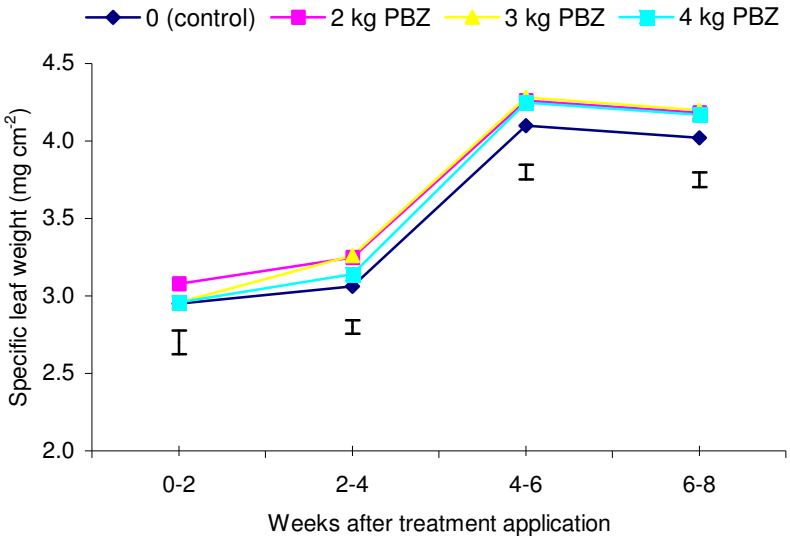

Figure 6.2 Specific leaf weight of potato as affected by rates of PBZ. The vertical bars represent least significant differences at P < 0.05

Crop growth rate of the control plants tended to be higher than that of the treated plants at comparable ontogenic stages (Figure 6.3). The maximum crop growth rates occurred in the interval 2-4 weeks after treatment, it slightly declined over the next two weeks, and sharply declined afterwards.

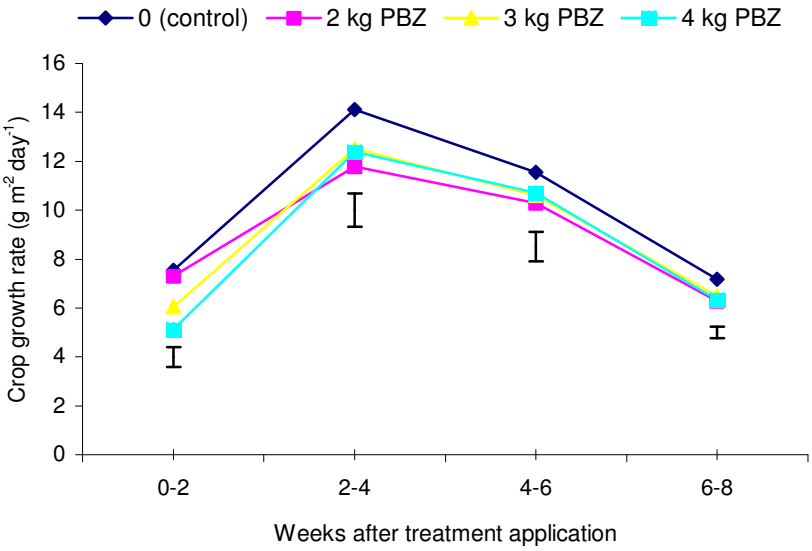

Figure 6.3 Effect of rates of PBZ on crop growth rate of potato. The vertical bars represent least significant differences at P < 0.05

PBZ enhanced the tuber growth rate (Figure 6.4). Up to 14 days after treatment, the control plants did not initiate tuber initials. Tuber growth rate increased to a peak of 5 g m^{-2} day^{-1} 4-6 weeks after treatment and showed a sharp decline afterwards.

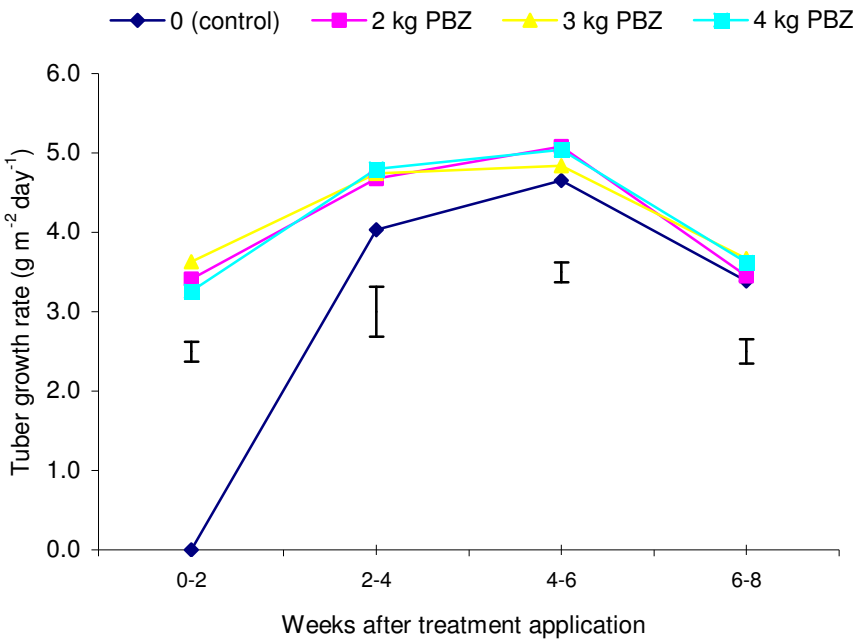

Figure 6.4 The effect of rates of PBZ on tuber growth rate of potato. The vertical bars represent least significant differences at P < 0.05

PBZ treatment slightly affected the net assimilation rate (Figure 6.5). During 0-2 weeks after treatment application, higher net assimilation rates were observed for plants which received 2 kg PBZ and for the control plants. During the 2nd and 3rd sampling periods net assimilation rate of 4 kg PBZ treated plants were slightly higher than the control. During the last sampling phase, no differences were observed among treatments with respect to net assimilation rate.

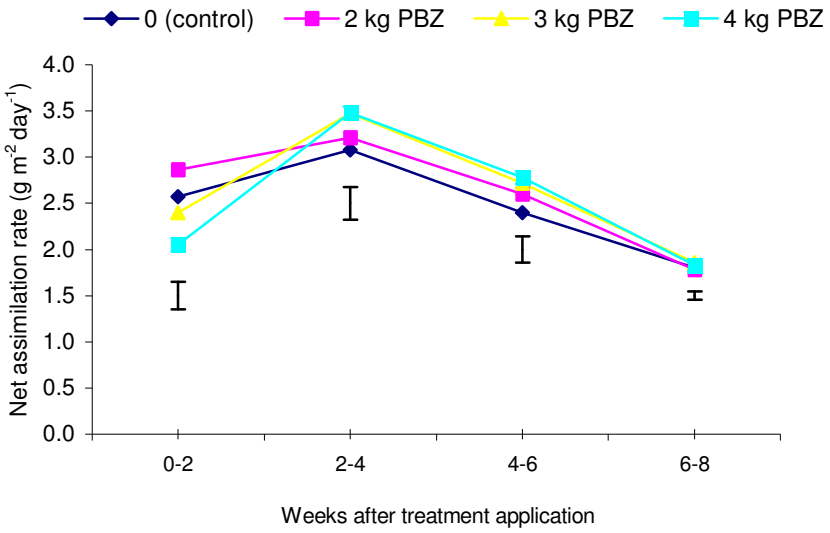

Figure 6.5 Net assimilation rate of potato as affected by rates of PBZ. The vertical bars represent least significant differences at P < 0.05

Dry matter allocation to the tubers was assessed by the partitioning coefficient (the ratio between tuber growth rate and crop growth rate). Although there was no significant difference at the third harvest, during the other harvesting periods PBZ increased the partitioning coefficient of the crop (Table 6.1).

Table 6.1 Partitioning coefficient of potato as influenced by different rates of PBZ

PBZ rate (kg a.i. ha^{-1})	PC			
	Week after treatment application			
	2	4	6	8
0 (control)	0.00c	0.29b	0.43a	0.47b
2	0.47b	0.40a	0.50a	0.54a
3	0.60a	0.38a	0.46a	0.56a
4	0.64a	0.38a	0.47a	0.56a
SEM	0.013	0.010	0.018	0.011

SEM: standard error of the mean.

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

6.5 DISCUSSION

The partitioning of carbon and nitrogen play a critical role in determining crop yield (Gifford & Evans, 1981). In most crops only part of the plant is utilized and hence the proportion of the total dry matter accumulated in the useful part of the plant is important, and will depend upon the sink strength of those organs. An understanding of the pattern of assimilate partitioning in potato is useful in determining potential yield, and to design strategies to increase tuber yield in the hot tropics and other areas where tuberization is poor. Triazoles are able to increase the partitioning of assimilates to tubers and roots and thereby increase yield (Fletcher *et al.,* 2000).

The mean leaf area index value of the control treatment (2-4 weeks after treatment) was approximately 4, which is the same order of LAI = 4.3 recorded 60 days after planting by Manrique (1989). PBZ reduced the leaf area index and this may be attributed to reduced GA activity in response to the treatment. It is postulated that reduced GA biosynthesis in response to PBZ treatment result in a reduction in cell proliferation, thus reducing leaf expansion. GA promotes cell division by stimulating cells in the G_1 phase to enter the S phase and by shortening the duration of S phase (Liu & Loy, 1976). Haughan *et al.* (1989) reported that the 2R configuration of PBZ retarded cell proliferation in celery. PBZ treatment decreased the length of wheat leaves by reducing cell length rather than cell number (Tonkinson *et al.,* 1995).

PBZ slightly increased leaf dry weight per unit area. Microscopic observations confirmed that treated plants had thicker leaves due to the induction of a thicker epicuticular wax layer, elongated and thicker epidermal cells, and palisade and spongy mesophyll tissues (Chapter 4). An increased leaf thickness in response to PBZ treatment has been confirmed in maize (Sopher *et al*., 1999), *Chrysanthemums* (Burrows *et al.,* 1992), and wheat (Gao *et al.,* 1987).

Net assimilation rates for the control treatment during the maximum tuber growth stage ranged from 2.5 to 3.5 g m^{-2} day^{-1}. This is lower than assimilation rates of 3 to 5 g m^{-2} day^{-1} reported for summer potato by Manrique (1989). The relatively lower net assimilation rate may be due to the poor adaptation of the cultivar used in the investigation to the prevailing high growing temperature.

The untreated plants exhibited a higher crop growth rate and a reduced tuber growth rate, while PBZ treated plants exhibited lower crop growth rates but a higher tuber growth rates. Higher leaf area is essential for higher biomass and tuber yield, however, in the current study it has been observed that the treated plants exhibited a higher tuber growth rate despite the reduced leaf area. This compensation could be due partly to enhanced net assimilation rate in response to the treatment. An increased tuber growth may also be attributed to an enhanced starch synthesis. From the microscopic investigation, it was clear that PBZ remarkably increased starch accumulation in the stem and root tissue of potato (Chapter 4). In the treated plants, numerous starch granules were observed in root and stem cortical cells as well as pith cells of the stem while cells in the control treatment were almost devoid of starch granules. It is evident from previous reports that high temperatures decrease tuber growth rate, reduce the partitioning of assimilates to the tubers and increase assimilation to other parts of the plant (Menzel, 1980; Struik *et al.,* 1989; Vandam *et al.,* 1996) which could be associated with increased GA activities

A reduction in crop growth rate and a concomitant increase in tuber growth rate increased the partitioning coefficient in PBZ treated plants. On the contrary, the untreated plants exhibited a lower partitioning coefficient due to excessive top growth and reduced tuber growth. Hence, it is reasonable to suggest that PBZ is effective in regulating top-tuber growth imbalance that

occurred regularly in the tropics. Manrique (1989) reported a reduced partitioning coefficient in summer grown potato that was due to an excessive top growth and reduced tuber growth.

6.6 CONCLUSION

The growth analyses demonstrated that PBZ reduced leaf area index, and crop growth rate, slightly increased net assimilation rate and partitioning of assimilates to the tubers, enhanced early tuberization and increase subsequent tuber growth of potato grown in a hot tropical lowlands. Consequently, the productivity of the crop improved.

CHAPTER 7

RESPONSE OF POTATO GROWN IN A HOT TROPICAL LOWLAND TO PACLOBUTRAZOL. III: TUBER ATTRIBUTES

7.1 ABSTRACT

The growth responses of potato to PBZ in the hot tropical conditions of eastern Ethiopia, was investigated in two field experiments during 2003. A month after planting PBZ was applied as a foliar spray or soil drench at rates of 0, 2, 3, and 4 kg a. i. PBZ per ha. PBZ increased tuber fresh mass, dry matter content, and specific gravity while promoting earlier tuber initiation and a reduction in tuber numbers. Root application of PBZ significantly increased crude protein content while both foliar and root PBZ applications extended the dormancy period. PBZ reduced the K and Mg contents of the tubers. Foliar applied PBZ increased the Ca content of tubers. Applying PBZ as a soil drench increased total tuber N. Both foliar and root applications increased tuber Fe content while reducing P levels. PBZ increased tuber yield, improved quality attributes such as dry matter content, crude protein content and Ca content, and extended the dormancy period of potatoes grown in the hot tropical lowlands of eastern Ethiopia.

Keywords: Crude protein**;** dormancy; dry matter; Ethiopia, nutrient composition; tuber quality, specific gravity; tuber yield

Publication based on this Chapter:

Tekalign, T. and Hammes, P. S. 2005. Response of potato grown in a hot tropical lowland to applied paclobutrazol. II: Tuber attributes. ***New Zealand Journal of Crop and Horticultural Science*** **33: 43-51.**

7.2 INTRODUCTION

Potato tuberization is a complex developmental process that requires the interaction of environmental, biochemical, and genetic factors (Kolomiets *et al.,* 2001). Low mean temperatures (15-19 °C) under a short photoperiod (12 h) are optimal for tuber initiation and early tuber growth (Vandam *et al.,* 1996). High temperatures delay the onset of tuber initiation and bulking, decrease absolute tuber growth rate and favour assimilate partitioning to the aboveground parts (Nagarajan & Bansal, 1990; Gawronska *et al.,* 1992; Vandam *et al.,* 1996; Jackson, 1999). Under cool temperatures and short photoperiods a transmissible signal is activated that triggers cell division and elongation in the sub-apical region of the stolons to produce tuber initials (Xu *et al.,* 1998; Amador *et al.,* 2001). In this signal transduction pathway, perception of appropriate environmental cues occurs in the leaves and is mediated by phytochrome and GA (Van den Berg *et al.,* 1995; Jackson & Prat, 1996).

Potatoes grown under high temperatures are characterized by high levels of endogenous GA (Vreugdenhil & Sergeeva, 1999) that have a delaying or inhibitory effect on tuberization (Abdella *et al.,* 1995; Vandam *et al.,* 1996). In addition, GA accumulation in tuber tissue can specifically impede starch accumulation (Booth & Lovell, 1972; Paiva *et al.,* 1983; Vreugdenhil & Sergeeva, 1999), inhibit the accumulation of patatin and other tuber specific proteins (Hannapel *et al.,* 1985; Vreugdenhil & Sergeeva, 1999), and in combination with other inhibitors regulate potato tuber dormancy (Hemberg, 1970).

In addition to the involvement of several endogenous growth substances, Koda *et al.* (1988) reported the existence of a specific tuberization factor that is produced or activated in the leaves and translocated to the stolons where it exerts its effect. Hammes & Nel (1975)

proposed that tuber formation is controlled by a balance between endogenous GA and tuber forming stimuli; for tuberization to occur the GA must be below a threshold level. This balance can be altered by the application of GA biosynthesis inhibitors such as 2-chloroethyl trimethyl ammonium chloride (CCC) (Menzel, 1980) and B-995 (Bodlaender & Algra, 1966). Recently, the *in vivo* and *in vitro* responses of potato to PBZ have been reported (Balamani & Poovaiah, 1985; Langille & Helper, 1992; Simko, 1994; Bandara & Tanino, 1995).

PBZ is a potent triazole plant growth regulator known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation path to block gibberellin synthesis (Rademacher, 1997). PBZ treatment increase root-to-shoot ratio (Pinhero & Fletcher, 1994; Yim *et al.*, 1997), increase partitioning of assimilates to economically important plant parts such as bulbs (Le Guen-Le Saos *et al.*, 2002, De Resende & De Souza, 2002). Although some researchers reported that PBZ enhances tuberization (Balamani & Poovaiah, 1985; Pelacho *et al.*, 1994; Simko, 1994), information is lacking regarding the effect of PBZ on the productivity of potato grown under tropical condition. It is proposed that PBZ enhances assimilate diversion to the tubers and thereby increase productivity and improve quality of potato grown under in hot tropical conditions. Accordingly, this chapter reports the effect of PBZ application methods and rates on tuber yield, quality, nutrient composition and dormancy of potato grown in the hot tropical lowlands of eastern Ethiopia.

7.3 MATERIALS AND METHODS

7.3.1 Site description

Details of the site are presented in Chapter 5.

7.3.2 Plant culture

Cultural methods are described in Chapter 5.

7.3.3 Treatments

The treatments that were applied are presented in Chapter 5.

7.3.4 Tuber parameters

Tuber initiation was recorded as occurring when the swollen portion of stolon tip attained a size of at least twice the diameter of the stolon (Ewing & Struik, 1992). For this purpose three plants per plot were tagged and tuber initiation monitored every second day. Tubers fresh mass and tuber numbers represent the average of 15 plants sampled per plot. At harvest, samples of about 5 kg tubers of all sizes from each plot were washed and dried. Tuber specific gravity was determined using the weight in air weight in water method (Murphy & Goven, 1959). For dry matter content determination, 3 kg tubers were pre-dried at 60 ºC for 15h and further dried for 3h at 105 ºC in a drying oven. Tuber dry matter content is the ratio between dry and fresh mass expressed as a percentage. Separate samples of 1 kg were dried at 60 ºC to constant mass, grounded and analysed for macro and micronutrient contents. Total nitrogen was determined using the Macro-Kjeldahl method (AOAC, 1984) and multiplied by a conversion factor of 6.25 to estimate tuber crude protein content (Van Gelder, 1981). Following wet-ash

digestion, phosphorus was determined by colorimetry, potassium by flame photometery, sulphur by turbidimetry, and calcium, magnesium, iron, copper, manganese and zinc by atomic absorption.

For dormancy evaluation, ten uniform (70-105 g) and healthy tubers were selected from each plot and labelled. The samples were stored in a naturally ventilated diffused light store in a randomised complete block design with three replications. The average daily minimum and maximum temperatures during the storage period were 13.6 ºC and 22.8 ºC, respectively and relative humidity ranged from 34 to 70%. The dormancy of a particular tuber was deemed to have ended when at least one 2 mm long sprout was present (Bandara & Tanino, 1995). The average dormancy period of the ten tubers was used to determine the dormancy period of a sample.

7.3.5 Statistical analysis

Described in Chapter 5.

7.4 RESULTS

Irrespective of the concentration, PBZ treated plants (Figure 7.1B, C and D) developed tuber initials about 17 days earlier than the control (Figure 7.1A). Regardless of the method of application, PBZ treatment increased tuber fresh mass, dry matter content, and specific gravity, and promoted early tuber initiation while reducing tuber number (Table 7.1). Fresh tuber yield per hill was increased from 195 g for untreated plants to 314 g by applying 3 kg a.i. PBZ. PBZ treatment reduced tuber numbers by about 21% as compared to the control.

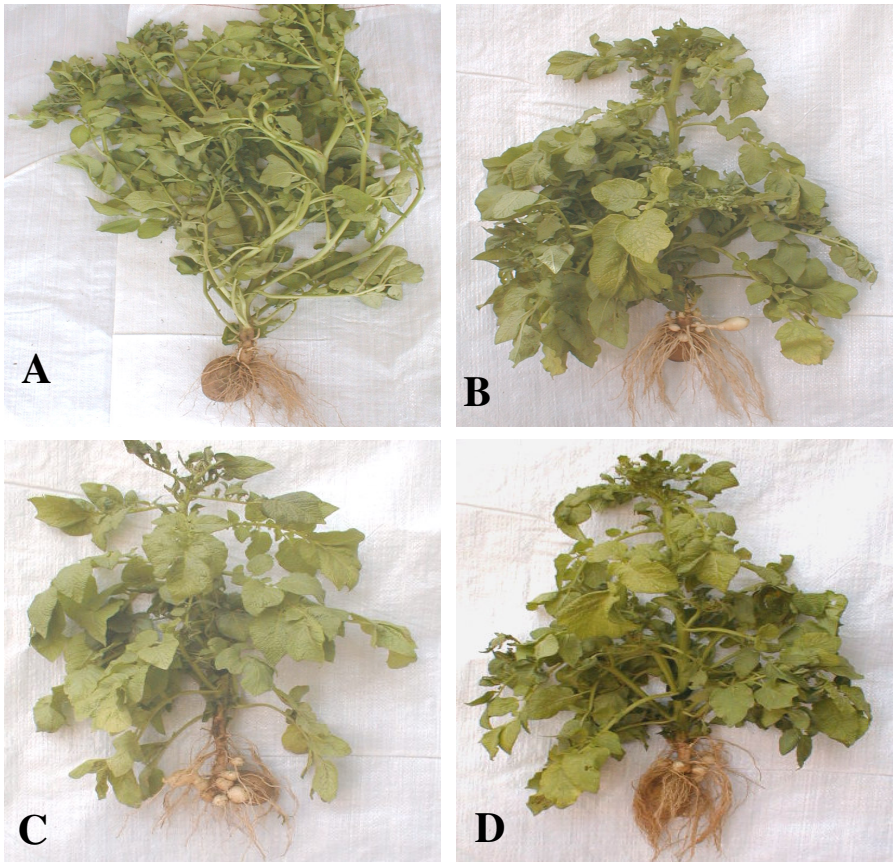

Figure 7.1 Potato plants two weeks after PBZ treatment at rates of 0 (A), 2 (B), 3 (C) and 4 kg a.i. ha^{-1} (D). The control plants had excessive top growth and no tuber formation, while the treated plants are characterized by reduced top growth and early tuberization

Table 7.1 Days to tuber initiation, fresh mass, number, dry matter content, and specific gravity of potato as affected by rates of PBZ

PBZ rate (kg a.i. ha^{-1})	Days to tuber initiation	Tuber fresh mass (g hill^{-1})	Tuber number (hill^{-1})	Dry matter content (%)	Specific gravity (g cm^{-3})
0 (Control)	54.0a	195c	7.6a	16.6c	1.061b
2	37.7b	300b	6.0b	17.5b	1.065a
3	37.3b	314a	6.1b	18.0a	1.068a
4	36.6b	305ab	6.0b	17.6ab	1.066a
SEM	0.48	3.26	0.09	0.09	0.004

SEM: standard error of the mean.

Means within the same column sharing the same letters are not significantly different ($P < 0.01$).

Average tuber fresh mass was negatively correlated with tuber number (r = - 0.98**). Tuber dry matter content varied from 16.6% (control) to 18.0% (3 kg a.i. PBZ), and specific gravity from 1.061 (control) to 1.068 (3 kg a.i. PBZ). Means of PBZ concentrations pooled over application method showed that application of 3 or 4 kg a.i PBZ increased dry matter content by about 7.2% and specific gravity was increased from 1.061 to mean value of 1.067 by PBZ.

Application method and PBZ concentration interacted significantly for tuber crude protein content and dormancy period (Table 7.2). Foliar spray of PBZ did not affect crude protein content, while applying 4 kg a.i. PBZ as a soil drench increased the protein content by about 12% compared to the control. Regardless of the concentration, foliar applied PBZ extended the tuber dormancy period by 17 days, while applying 3 or 4 kg a.i. PBZ as a soil drench prolonged dormancy by about 20 days.

Table 7.2 The effect of application method and rate of PBZ on the crude protein content and dormancy period of potato

Application method	PBZ rate (kg a.i. ha^{-1})	Crude protein (% DM)	Dormancy period (days)
Foliar spray	0 (control)	11.67b	45.64c
	2	11.46b	61.99b
	3	11.88b	62.63b
	4	11.67b	63.32b
Soil drench	0 (control)	11.88b	45.33c
	2	11.67b	63.27b
	3	11.88b	65.89a
	4	13.34a	65.08a
	SEM	0.08	0.39

SEM: standard error of the mean.

Means within the same column sharing the same letters are not significantly different ($P < 0.01$).

The tuber mineral composition was affected by both method of application and rate of PBZ (Table 7.3). Irrespective of the concentration, PBZ treatment reduced K and Mg contents of the tubers while Ca, S, Cu and Zn concentrations were unaffected. Compared to soil drench, foliar spray reduced K content but increased the Ca content of the tubers.

Table 7.3 Potassium, calcium, magnesium, sulphur, copper and zinc concentrations (dry matter basis) in potato tubers as affected by application method and concentration of PBZ

Treatment	K (%)	Ca (%)	Mg (%)	S (%)	Cu (ppm)	Zn (ppm)
Foliar spray	3.05b	0.14a	0.16a	0.52a	17.33a	34.16a
Soil drench	3.15a	0.13b	0.16a	0.53a	14.83a	34.75a
SEM	0.02	0.004	0.002	0.02	1.42	2.74
0 (control)	3.44a	0.13a	0.18a	0.55a	17.50a	31.33a
2 (kg a.i. ha^{-1})	2.98b	0.13a	0.15b	0.58a	15.50a	34.50a
3 (kg a.i. ha^{-1})	2.99b	0.13a	0.15b	0.50a	14.50a	40.33a
4 (kg a.i. ha^{-1})	2.99b	0.14a	0.15b	0.48a	16.83a	31.67a
SEM	0.03	0.005	0.004	0.03	2.01	3.88

SEM: standard error of the mean.

Means for the same main effect within the same column sharing the same letters are not significantly different ($P < 0.01$).

A significant interaction between application method and concentration of PBZ was observed with respect to N, P, Fe, and Mn content of the tubers (Table 7.4). Foliar spray of any PBZ concentration did not increase N content, while application of 4 kg a.i. PBZ as a soil drench increased N concentration by 12%. Irrespective of the rate, foliar application and soil drenching of PBZ reduced P concentration by about 11 and 6% respectively compared to the check. Foliar spray of 3 or 4 kg a.i. PBZ increased tuber Fe content by 64%, while drench applications of 2 or 4 kg a.i. increased Fe content by about 54% over the control. Treating plants with 3 kg PBZ as a foliar spray increased Mn concentration by about 52%, while soil

drenching with 3 or 4 kg PBZ increased the Mn content by approximately 68% as compared to the control.

Table 7.4 The effect of application method and rate of PBZ on total nitrogen, phosphorus, iron and manganese content of potato tubers. Values are calculated on dry matter basis

Application method	PBZ rate (kg a.i. ha^{-1})	N (%)	P (%)	Fe (ppm)	Mn (ppm)
Foliar spray	0 (control)	1.87b	0.47a	60.33c	7.00d
	2	1.83b	0.41d	57.33c	6.67d
	3	1.90b	0.43bcd	102.00a	10.67ab
	4	1.83b	0.42cd	95.67ab	8.67bcd
Soil drench	0 (control)	1.90b	0.47a	70.67bc	7.33cd
	2	1.87b	0.44bc	101.33a	10.33bc
	3	1.90b	0.43bcd	91.67ab	11.00ab
	4	2.13a	0.45ab	115.67a	13.67a
	SEM	0.03	0.004	6.62	0.73

SEM: standard error of the mean.

Means of the same main effect within the same column sharing the same letters are not significantly different ($P < 0.01$).

7.5 DISCUSSION

For optimal yield and quality, potatoes prefer cool temperate climates with low mean temperatures and a short photoperiod (Vandam *et al.,* 1996). Nevertheless, potato has been produced in many tropical climates under high temperatures, resulting in yield reductions and quality deterioration. This is partly attributed to the synthesis of high levels of endogenous GA, which delays or inhibits tuber initiation, reduces partitioning of assimilates to the tubers, and impedes the synthesis of starch and tuber specific proteins. This study investigated the effect

of applied PBZ on the tuber yield and quality of potato grown in hot tropical conditions of eastern Ethiopia to use as a possible intervention.

Crop yield is a function of canopy size (LAI) for intercepting solar radiation, the persistence of photosynthetically active leaf area (LAD), and the efficiency of net gain of assimilates (NAR). In spite of a reduction in LAI and total biomass (Chapter 6), PBZ increased tuber growth and resulted in about a 57% yield advantage over the control, which may be linked to early tuberization, increased leaf chlorophyll content, enhanced rate of photosynthesis, and delaying the onset of senescence (Chapter 5). The reduction in tuber numbers may be attributed to a decline in stolon number in response to a decrease in GA biosynthesis, but no specific observations in this regard were made. The involvement of GA in regulating stolon numbers through stolon initiation was reported by Kumar & Wareing (1972). Frommer & Sonnewald (1995) reported that the competition among tuber initials reduces the final tuber number. The strong negative association between tuber fresh mass and number signify that PBZ increased tuber yield by increasing tuber size. In agreement with this, Balamani & Poovaiah (1985) and Simko (1994) reported increased tuber dry weight per plant in response to PBZ, although it was not clear if the increase was a consequence of tuber size. In contrast, Bandara & Tanino (1995) reported that PBZ nearly doubled the number of tubers per plant without affecting the total fresh weight of the tubers. High temperature increases GA biosynthesis that reduces tuber sink strength to attract photoassimilates and may cause yield reduction (Booth & Lovell, 1972). Krauss (1978) reported that GA: abscisic acid (ABA) ratio controls tuberization and subsequent tuber growth; relatively higher GA levels reduce or stop tuber growth, while higher ABA levels promote tuber growth.

PBZ increased the dry matter content and specific gravity of the tubers. This may be attributed to reduced tuber GA levels with a subsequent increase in sink strength, enhancing starch synthesis and deposition. Booth & Lovell (1972) reported reduced sink strength of tubers due to GA_3 accumulation in tuber tissue. Under conditions favourable for tuberization the activity of enzymes involved in starch biosynthesis such as ADPG-pyrophosphorylase, starch phosphorylase and starch synthase increase (Visser *et al.,* 1994; Appeldoorn *et al.,* 1997). Mares *et al.* (1981) observed that exogenous application of GA_3 to growing tubers substantially reduced the activity of ADPG-pyrophosphorylase, while the activity of starch phosphorylase remained more or less constant. Similarly, Booth & Lovell (1972) observed that application of GA_3 to potato shoots reduced export of photosynthates to the tubers, decreased starch accumulation, increased sugar levels and resulted in cessation of tuber growth.

PBZ increased tuber crude protein content, probably due to blocking of GA biosynthesis that is known to inhibit tuber protein synthesis. The increased total nitrogen concentration in tubers from PBZ treated plants may be due to an increased uptake of nitrogen from the soil and/or remobilisation of nitrogen from other plant parts to the tubers. Park (1990) and Vreugdenhil & Sergeeva (1999) reported the negative effects of GA_3 on the synthesis of patatin and other tuber specific proteins. The involvement of GA in the regulation of potato tuber starch and protein synthesis, along with a strong association between starch and protein content is reported by Paiva *et al.* (1983).

PBZ extended tuber dormancy, probably by blocking GA biosynthesis and reducing ABA catabolism (Rademacher 1997) which could result in a low GA to ABA ratio in developing tubers. Dogonadze *et al.* (2000) observed that exogenous GA_3 treatment promoted tuber

sprouting by enhancing RNA and DNA synthesis, and Hemberg (1970) reported an inhibitory effect of ABA to tuber sprouting through inhibited RNA and DNA synthesis. The regulatory effects of GA and ABA on RNA and DNA synthesis are probably the major contributors to delayed sprouting (Shik & Rappaport, 1970). It is suggested that the ratio of GA to ABA in the tuber is the most probable control mechanism of potato dormancy. Harvey *et al.* (1991), Simko (1994) and Bandara & Tanino (1995) also reported that PBZ treatment extended the dormancy period of the tubers.

PBZ influenced the anatomy and morphology of roots as described in Chapter 4 and this might have altered mineral uptake and hence, tuber nutrition. PBZ increased potato tuber yield by increasing tuber size and the observed reduction in some nutrient concentrations may be due to a dilution effect. Reports on the effects of PBZ on mineral element content are not consistent and mainly refers to fruit crops. For instance, Yelenosky *et al.* (1995) observed that leaves from PBZ treated citrus seedlings had higher concentration of N, Ca, B, Fe, and Mn. Wang *et al.* (1985) reported that PBZ treatment increased leaf N, P, K, Ca, Mg, Mn, Ca, Zn, and Sr concentration in apple while the contents of Fe, Si, and Pb were unaffected. In contrast, Wieland & Wample (1985) reported that PBZ did not influence N, P, K, and Mg content of apple leaves. It was also reported that the mineral composition of apple fruit was unaffected by PBZ treatment (Steffens *et al.,* 1985). Very recently, Yeshitela *et al*. (2004) reported that PBZ increased Mg, Cu, Zn, and Fe content of mango leaves without affecting the concentration of N, P, K, and Ca.

7.6 CONCLUSION

PBZ increased tuber yield and quality indicating its potential to improve potato production in the tropics. However, detailed investigations are essential to identify the correct time and rate of application, and analyse risks in terms of human health and environmental pollution. Prolonging tuber dormancy period with PBZ may be useful for the potato industry, particularly to reduce untimely sprouting of potato cultivars having a short dormancy period. However, the effect of residual PBZ on the performance the next generation must be investigated. For all the parameters considered, little significant difference was observed between foliar spray and soil drench applications. For ease of application and to reduce soil pollution foliar spray is suggested as the method of application.

CHAPTER 8

GROWTH AND PRODUCTIVITY OF POTATO AS INFLUENCED BY CULTIVAR AND REPRODUCTIVE GROWTH: I. STOMATAL CONDUCTANCE, RATE OF TRANSPIRATION, NET PHOTOSYNTHESIS, AND DRY MATTER PRODUCTION AND ALLOCATION

8.1 ABSTRACT

The effect of cultivar and reproductive growth on leaf gas exchange, water relations, dry matter production and allocation in potato was the subject of investigation. Debudded, flowering and fruiting plants of cultivars Al-624, Al-436, CIP-388453-3(A) and CIP-388453-3(B) were evaluated under field condition of a sub-humid tropical highland of Ethiopia during 2003. Cultivars exhibited differences with respect to leaf stomatal conductance, rate of transpiration and photosynthetic efficiency. Cultivars Al-624 and CIP-388453-3(A) showed higher stomatal conductance and rate of leaf transpiration than CIP-388453-3(B) and Al-436. Cultivar CIP-388453-3(A) exhibited higher net photosynthesis than Al-624 while Al-436 is intermediate. Fruiting plants exhibited higher leaf stomatal conductance and higher rate of leaf transpiration and net photosynthesis. Fruit development promoted early plant maturity, and without affecting total dry matter production it reduced the amount partitioned to the leaves, stems, and tubers.

Keywords: Assimilate partitioning, berry set, Ethiopia; flowering, genotype, potato

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8.2 INTRODUCTION

Potato (*Solanum tuberosum* L.) is an annual crop that can be propagated vegetatively from seed tubers or sexually using botanical seed. Flowering in potato occurs in various degrees depending on the cultivar and environmental conditions (Sadik, 1983; Lozaya-Saldana, 1992). Its expression is influenced by internal and external factors, including source-sink equilibrium, hormonal balance, physiological maturity and photoperiod (Lozaya-Saldana & Miranda-Verlazguez, 1987; Lozaya-Saldana, 1992). Generally, *Solanum tuberosum* ssp *andigena* flowers regardless of the day length although flowering increases under short days, while *Solanum tuberosum* ssp *tuberosum* does not flower under short days (Sadik, 1983).

In Ethiopia most of the cultivars produce profuse flowers and some of them set berries. For commercial potato production seed tubers are mainly used and the use of true potato seed is limited to breeding activities at research institutions. Fruit formation is an undesirable quality because the berries have no subsidiary uses.

The assimilation of dry matter and its distribution within the plant are important processes determining crop productivity. A higher investment to the vegetative organs may give high total biomass and a relatively low proportion may be used for the production of storage organs, especially if the maintenance requirements of the vegetative organs are high (Van Heemst, 1986). Studying the pattern of dry matter allocation amongst plant parts, the variability of this pattern among cultivars, and the effect of environmental conditions on the process can help in maximizing productivity and selection of a cultivar for a particular purpose. The presence of plant organs with a net demand for assimilates can strongly influence the pattern of dry matter production and distribution (Gifford & Evans, 1981). Developing fruit has a considerable effect on the growth of other plant organs in such a way that with increasing fruit load the growth of

roots, shoots and leaves are often reduced (Marcelis, 1992; Heuvelink, 1997). Net photosynthesis, gauged either by growth analysis or by direct measurements of gas exchange, is higher in plants with actively growing fruit (Lenz, 1979; Eckstein *et al*., 1995). The effect of flowering and berry set on the photosynthetic efficiency and rate of transpiration of potato has not been well investigated. There are reports indicating that the distribution of assimilates among sinks is primarily regulated by the sinks themselves (Ho *et al.*, 1989; Marcelis, 1996).

The suppressing effects of reproductive growth on vegetative growth have been reported in cucumber (Marcelis, 1992), tomato (Heuvelink, 1997), banana (Eckstein *et al*., 1995), dandelion (Letchamo & Gosselin, 1995) and chestnut (Famiani *et al*., 2000). Very little research has been done regarding the effect of reproductive growth on biomass production and allocation in potato. This chapter reports on the effect of cultivar and reproductive growth on leaf stomatal conductance, rate of transpiration, net photosynthesis, dry matter production and assimilate distribution in potato. The effect of cultivar and reproductive growth on tuber yield and yield components, specific gravity, dry matter content and nutrient compositions is presented in the following chapter (Chapter 9).

8.3 MATERIALS AND METHODS

8.3.1 Experimental site description

The study was conducted during February to June 2003 on the Research Farm of Alemaya University, Ethiopia. The experimental site is located at 42° 3'E longitude, 9° 26'N latitude and at an altitude of 1980 m above sea level. It is situated in the semi-arid tropical belt of eastern Ethiopia and characterized by a sub-humid climate with an average annual rainfall of about 790 mm, annual mean temperatures of 17 ºC with mean minimum and maximum temperatures of 3.8 and 25 ºC, respectively. During the study period, the mean maximum temperature was 26 ºC (ranging from 20.5 to 29 ºC) and minimum temperature 11.4 ºC (ranging from 7.8 to 16.4 ºC). During the growing period a total of 177 mm precipitation was received and supplementary irrigation was applied. Mean sunshine hours were 9.7 per day, along with a relative humidity of 41% (ranging from 19 to 71%). The soil of the experimental site is a well-drained deep alluvial that contains 14 g kg^{-1} organic carbon, 1.4 g kg^{-1} total nitrogen, 0.01 g kg^{-1} available phosphorus, 0.47 g kg^{-1} total potassium, and a pH of 7.2.

8.3.2 Cultivars

To obtain a range of genotypes from comparatively light to profusely blooming types, and from light to heavy fruit setting, cultivars with different floral and berry development behaviour were selected. The four selected cultivars were CIP-388453-3(A), CIP-388453-3(B), Al-624, and Al-436, all with a determinate growth habit (Figure 8.1).

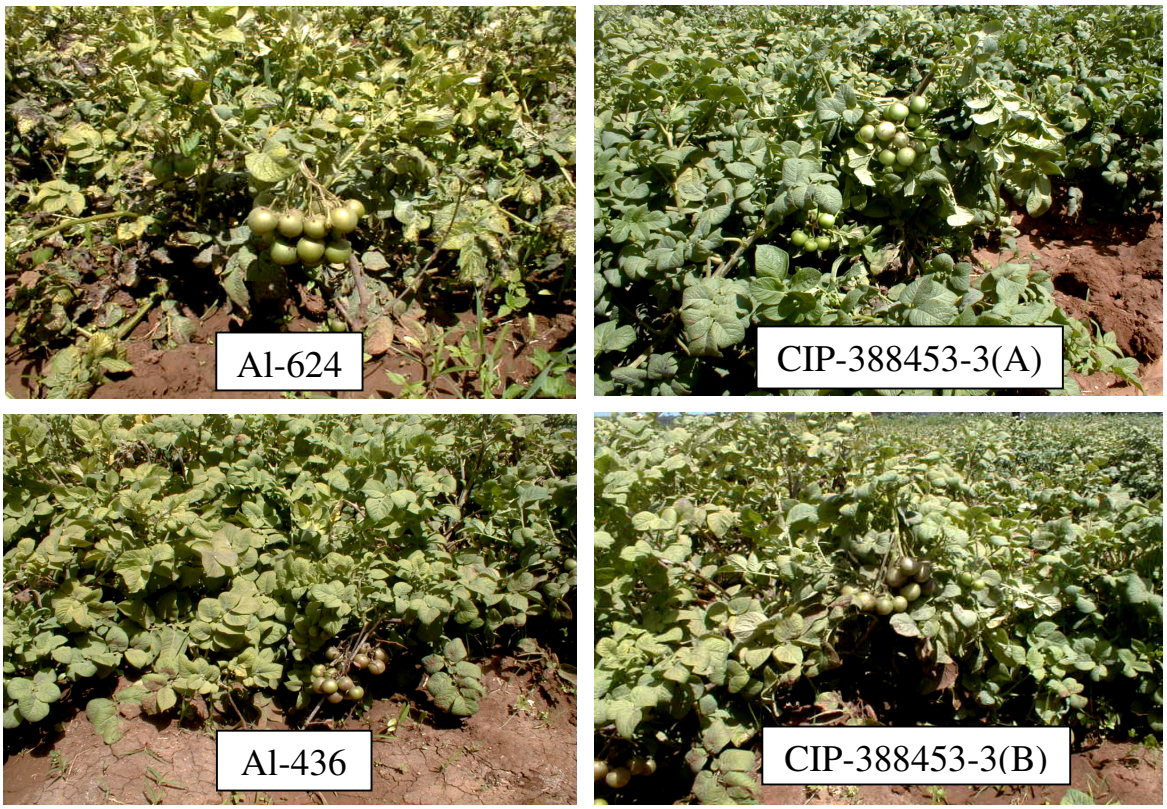

Figure 8.1 Cultivars used for the study

8.3.3 General field procedure

The experimental plots were arranged in a split-plot design in a randomised complete block design replicated three times. The four cultivars were assigned to the main plots and the three reproductive growth manipulation treatments to the sub-plots. Forty-nine medium sized and well-sprouted tubers of each cultivar were planted in seven rows of a sub-plot (size = 11.025 m^2) at a spacing of 75 x 30 cm. Sub-plots within the main plots were arranged continuously without board rows, and the end plots were bordered by two rows of potato plants. Phosphorus was applied as diammonium phosphate at planting time at a rate of 150 kg P ha^{-1} and nitrogen was side dressed after full emergence at a rate of 100 kg N ha^{-1} in the form of urea. All other cultural practices were applied according to the regional recommendation (Teriessa, 1997). No major disease and insect pest incidences were encountered.

8.3.4 Treatments

The study was designed to grow plants of the four cultivars by providing the following three different types of treatments:

1. **Non-flowering plants (debudded plants)**: Flower clusters were nipped off at bud emergence stage at two-day intervals (Figure 8.2A).

2. **Flowering plants**: The plants were permitted to flower but not to set fruit. The flowers were removed after anthesis. This process was repeated every two days (Figure 8.2B).

3. **Fruiting plants (control)**: Plants were allowed to flower and set berries (Figure 8.2C).

Figure 8.2 Non-flowering (A), flowering (B), and fruiting (C) treatments applied to cultivar CIP-388453-3(B)

8.3.5 Data recorded

Gas exchange

Two, four, and six weeks after debudding commenced, leaf stomatal conductance, rate of transpiration and net photosynthesis were measured using a portable LCA-4 photosynthesis system (Analytical Development Company, Bio Scientific Ltd., UK). From each sub-plot, three plants were randomly selected and the measurements were taken on the terminal leaflets of the three youngest fully expanded leaves. During the measurements the photon flux density incident at the level of the leaf in the cuvette ranged between 1995 and 2644 $\mu molm^{-2}s^{-1}$. The external carbon dioxide concentration varied between 342 and 354 $\mu molmol^{-1}$. Since the cultivars varied with respect to days to flowering, gas exchange measurements were taken on different days for the different cultivars.

Assimilate partitioning

Eight weeks after debudding of a specific cultivar commenced, three randomly selected plants per sub-plot were sampled and separated into different parts. The samples were oven dried at 72 °C to a constant mass. Dry matter partitioning to the different organs was expressed as a percentage of the total biomass. Days elapsed to reach physiological maturity were recorded when about 50% of the leaves senesced.

8.3.6 Statistical analysis

The analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C 1991). Means were compared using least significant differences (LSD) test at 5% probability level. Correlations between parameters were computed when applicable.

8.4 RESULTS

The cultivars differed greatly with respect to the degree of berry production. The ranking of the cultivars in decreasing order of fresh berry mass is Al-624 (275 g hill^{-1}), CIP-388453-3(B) (226 g hill^{-1}), Al-436 (209 g hill^{-1}), and CIP-388453-3(A) (81 g hill^{-1}). Cultivar Al-624 produced 26 berries per hill, followed by CIP-388453-3(B), Al-436, and CIP-388453-3(A) with respective mean berry numbers of 22, 19, and 14.

Leaf stomatal conductance was influenced by cultivar and pruning treatments independently. Cultivar means pooled over treatments showed that during all measurements, the stomatal conductance of Al-624 and CIP-388453-3(A) was higher than that of Al-436 with CIP-388453-3(B) intermediate (Figure 8.3A). At all measurement phases, fruiting plants had consistently higher stomatal conductance than flowering and non-flowering plants (Figure 8.3B).

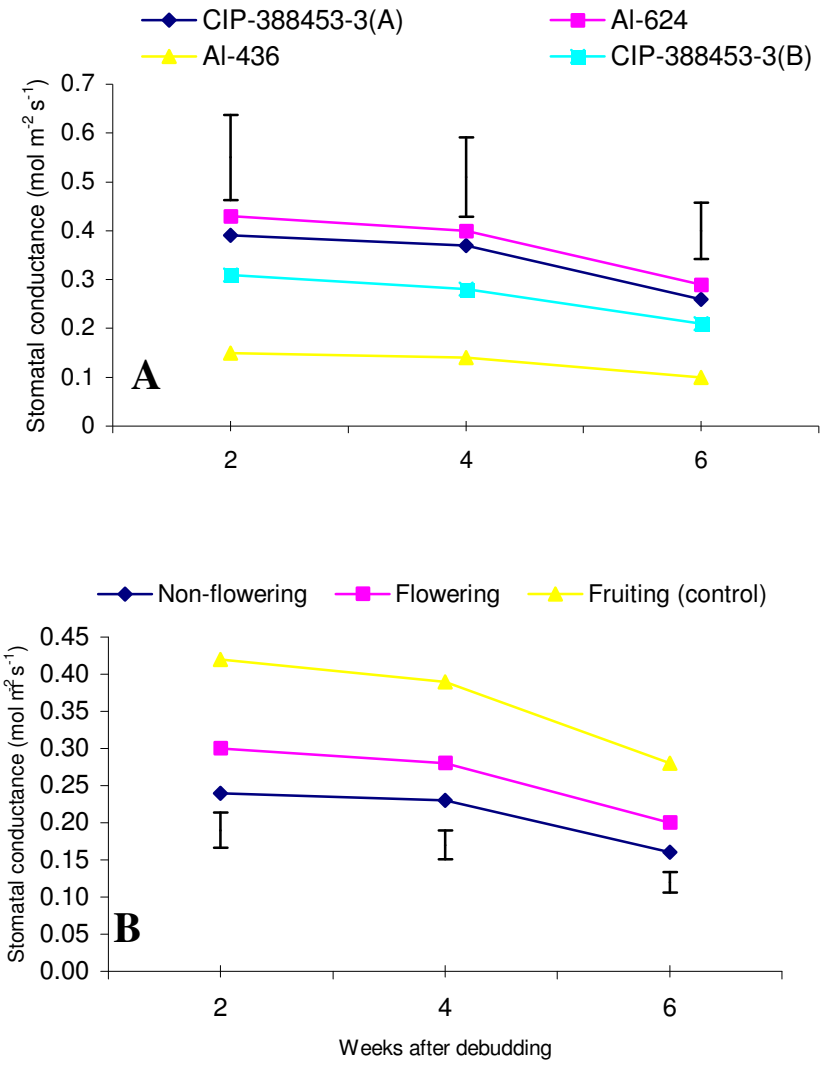

Figure 8.3 Leaf stomatal conductance of potato as affected by cultivar (A) and reproductive growth (B). The vertical bars represent least significant differences at P < 0.05

Distinct differences among cultivars were exhibited with respect to rate of leaf transpiration as shown in Figure 8.4A. The leaf transpiration rate of Al-624 and CIP-388453-3(A) was higher than Al-436 and CIP-388453-3(B). During the three observation periods (two, four, and six weeks after debudding) higher leaf transpiration rates were recorded on fruiting plants than on flowering and non-flowering plants (Figure 8. 4B).

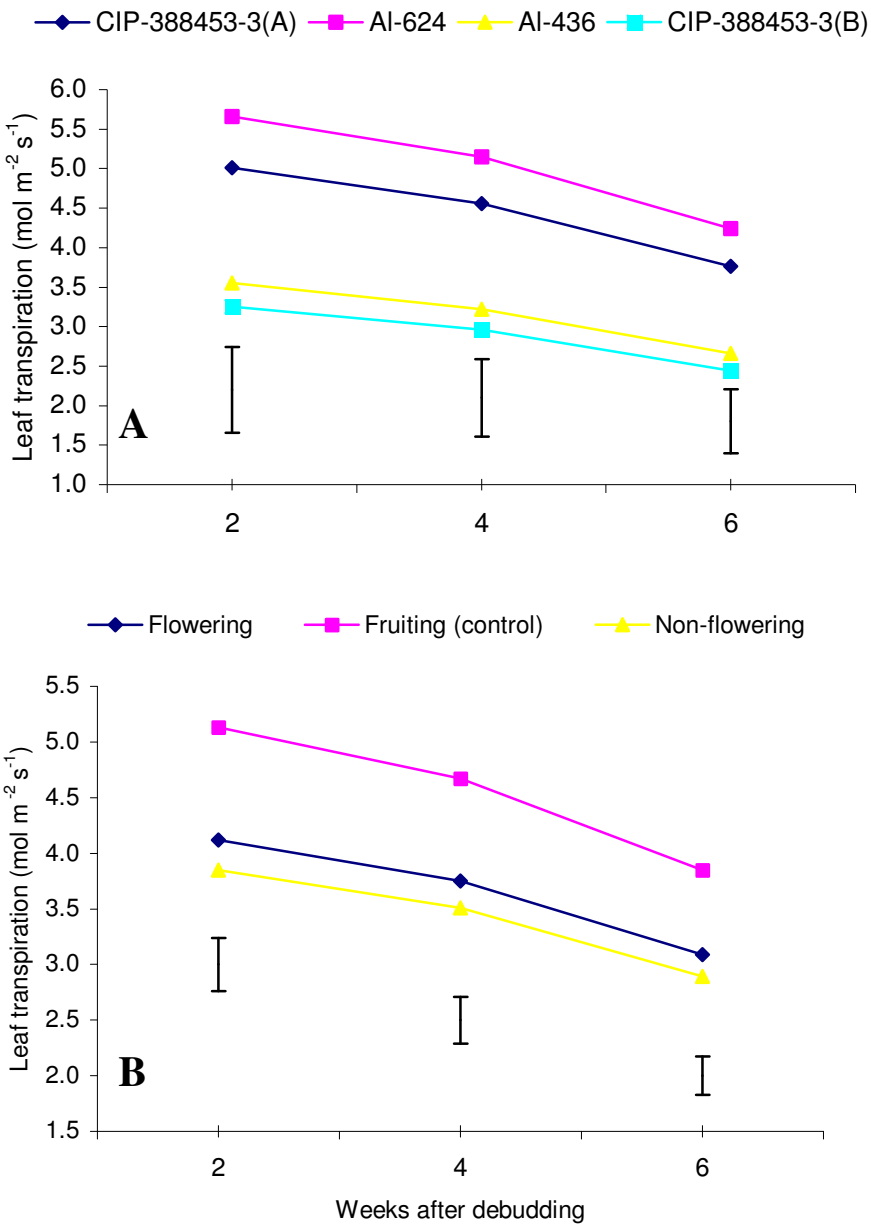

Figure 8.4 Leaf transpiration of potato as influenced by cultivar (A) and reproductive growth (B). The vertical bars represent least significant differences at P < 0.05

During the observation periods, the net photosynthetic rate of cultivar CIP-388453-3(A) was consistently higher than Al-624, with Al-436 intermediate (Figure 8.5A). Like stomatal conductance and rate of transpiration, the fruiting plants had higher photosynthetic rates than the other two groups (Figure 8.5B). The overall trend showed that leaf stomatal conductance, rate of

transpiration and net photosynthesis tended to decline from two weeks after debudding until last monitoring, six weeks after debudding..

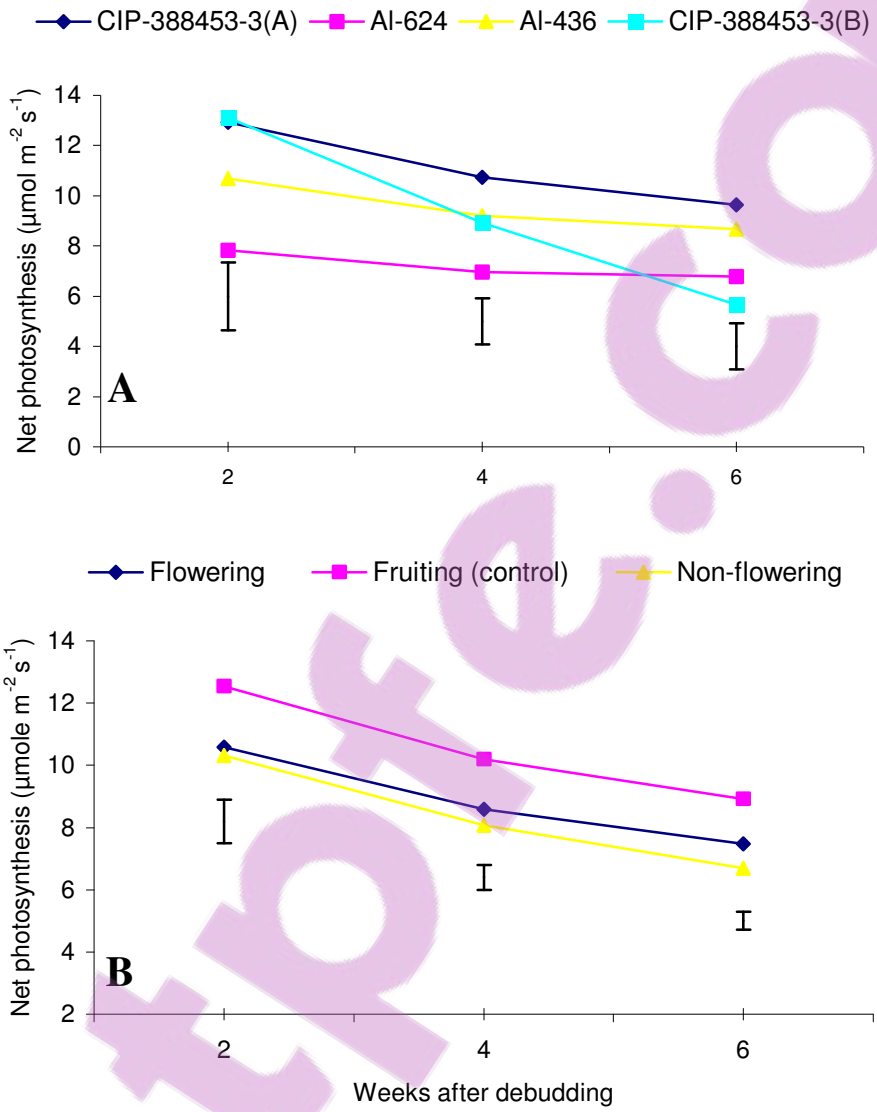

Figure 8.5 Net photosynthesis of potato as influenced by cultivar (A) and reproductive growth (B). The vertical bars represent least significant differences at P < 0.05

The dynamics of growth as measured by dry matter accumulation two, four, six and eight weeks after debudding showed that cultivar CIP-388453-3(A) produced a higher total biomass than Al-436, CIP-388453-3(B), and Al-624 (Figure 8.6A). Flowering and berry set slightly but significantly affected total biomass production at all sampling periods (Figure 8.6B).

During the second sampling period, debudded plants produced the highest biomass (223 g), followed by fruiting (216 g) and flowering plants (209 g). During the third and fourth sampling period, the fruiting and debudded plants produced a higher biomass than the flowering plants.

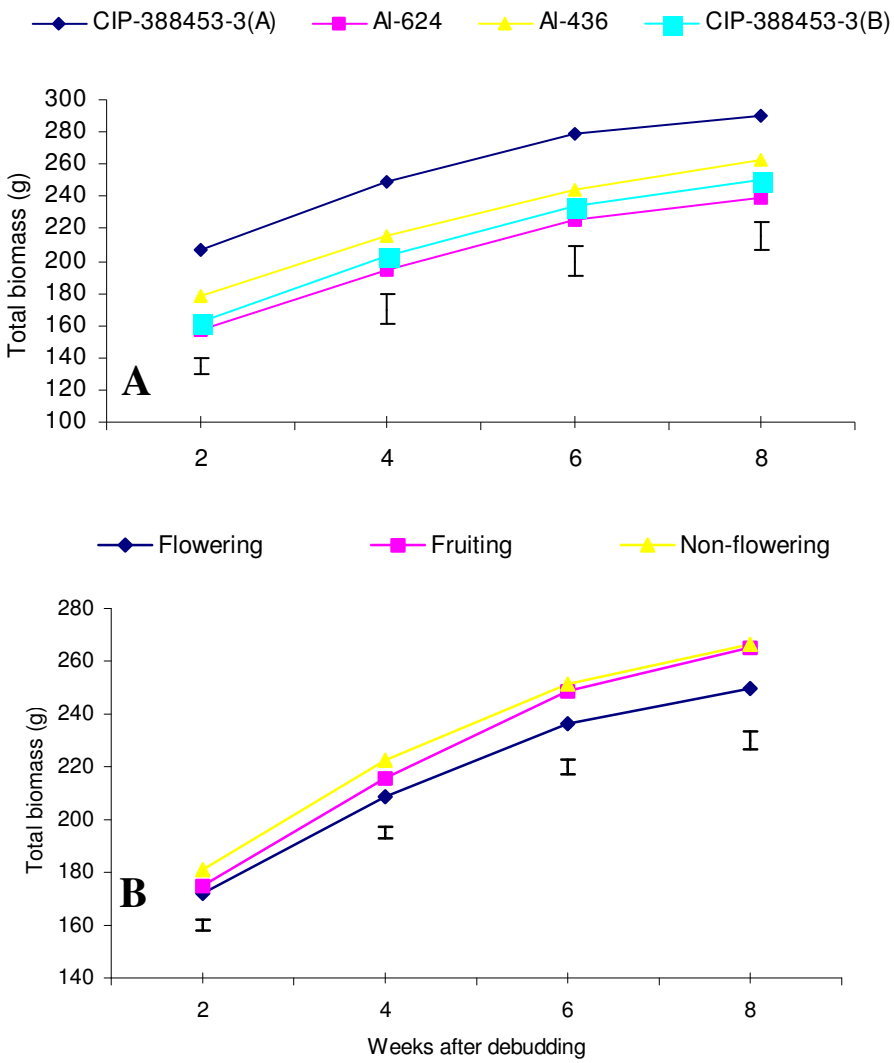

Figure 8.6 Total biomass yield of potato as affected by cultivar (A) and reproductive growth (B). The vertical bar represents least significant differences at P < 0.05

The fraction of dry matter partitioned amongst plant components eight weeks after debudding is presented in Figure 8.7A. Cultivar Al-624 had diverted more dry matter to the leaves than CIP-388453-3(A) and CIP-388453-3(B), while Al-436 was intermediate. CIP-388453-3(A) partitioned a larger fraction of the dry mass to the stems than the other cultivars. Al-624 and CIP-

388453-3(B) partitioned more assimilates to the developing fruit than CIP-388453-3(A) and Al-436. Of the total carbon fixed, the cultivars partitioned about 4% to the roots. CIP-388453-3(A), Al-436, and CIP-388453-3(B) allotted about 36% of the total dry matter to the tubers, which is higher than that partitioned by Al-624 (31%). The effect of reproductive growth on assimilate partitioning is indicated in Figure 8.7B. Fruiting plants utilised 9% of the assimilates for the production of berries, and partitioned less to the leaves, stems, and tubers than flowering and non-flowering plants.

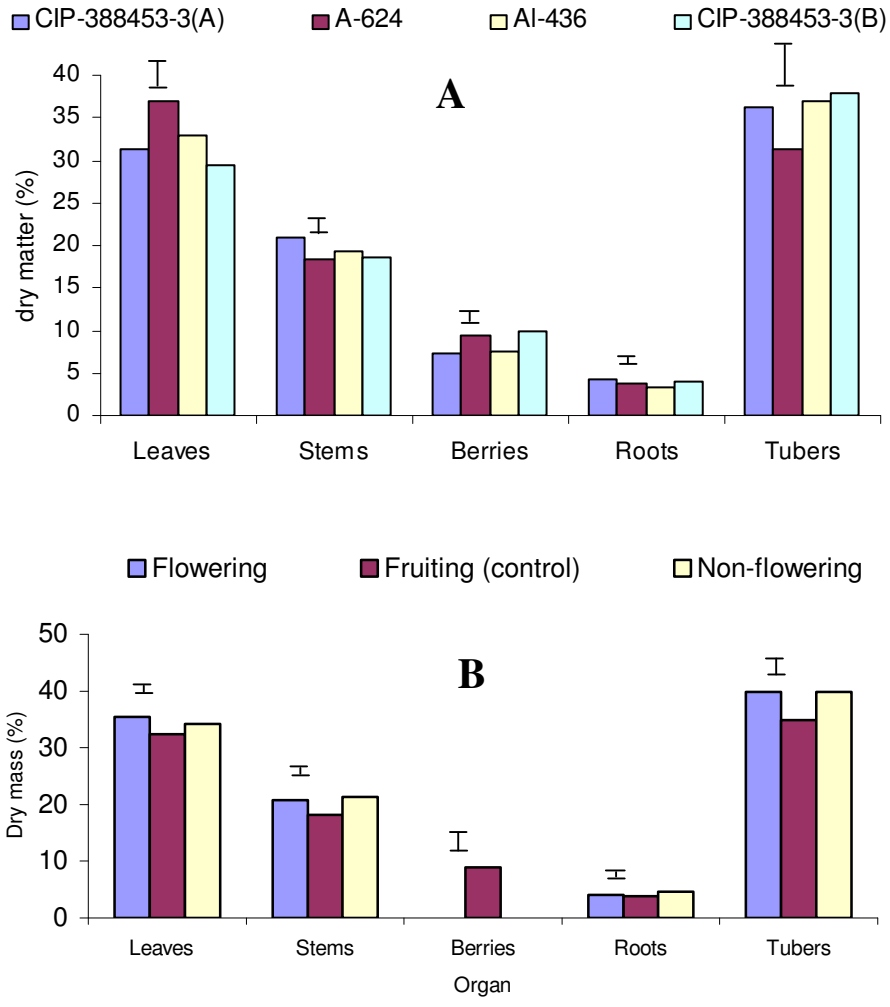

Figure 8.7 Dry matter distribution (% of the total dry mass) among organs of potato as influenced by cultivar (A) and reproductive growth (B) (eight weeks after flower bud initiation). The vertical bar represents least significant differences at P < 0.05

A significant variation in days to maturity occurred among cultivars (Figure 8.8A). Cultivar CIP-388453-3(A) required about 110 days to reach maturity. On the other extreme, cultivar Al-624 attained maturity within 92 days after planting. The presence of reproductive growth accelerated the onset of senescence in potato (Figure 8.8B). Fruiting plants showed the onset of senescence a week before the non-flowering plants.

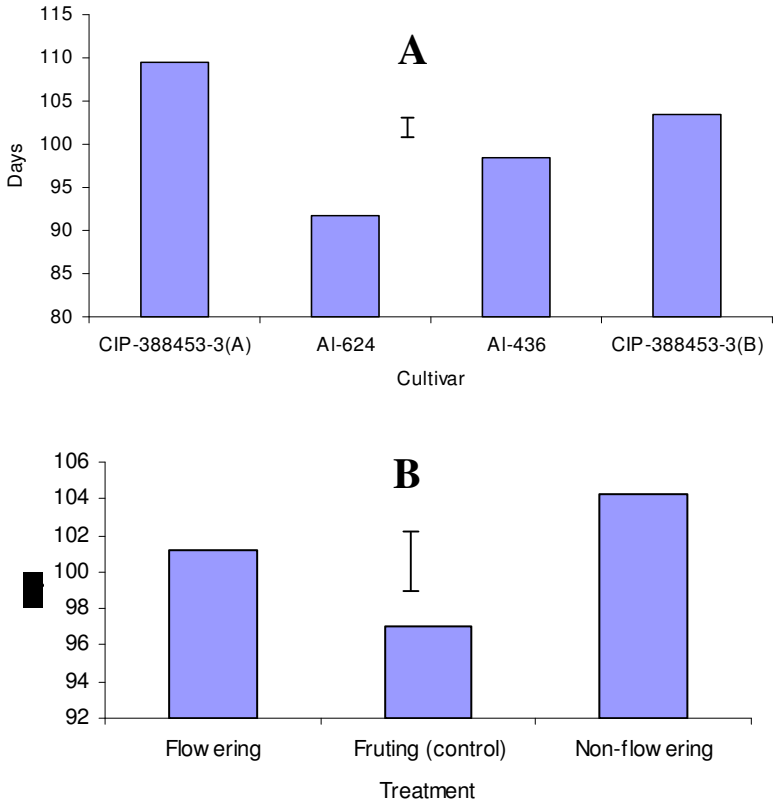

Figure 8.8 Physiological maturity of potato as affected by cultivar (A) and reproductive growth (B). The vertical bar represents least significant differences at P < 0.05

8.5 DISCUSSION

The cultivars Al-624 and CIP-388453-3(A) exhibited higher leaf stomatal conductance and rates of transpiration than the other cultivars. Dwelle *et al*. (1981b) reported the existence of genotype differences in potato regarding stomatal diffusive resistance and stomatal conductance. This may be linked to the variation in abscisic acid accumulation, which is an important trait to improve yield in a water-limited environment. The presence of berries increased leaf stomatal conductance and rate of transpiration. It is postulated that the developing fruit decrease the level of endogenous ABA and thereby increase leaf stomatal conductance, and concomitantly the rate of transpiration. ABA regulates the opening and closing of stomata (Salisbury & Ross, 1992). ABA causes stomatal closure and stimulates the uptake of water into roots (Hartung & Jeschke, 1999). Luckwill (1975) reported that leaves in close proximity to developing fruit contain much less ABA and have lower stomatal resistance than leaves more distant from the fruit. Similarly, Loveys & Kriedmann (1974) from their investigations with many plant species reported an increased level of ABA and phaseic acid in response to fruit removal. Removing the growing pod in soybean decreased the level of IAA-esters moving to the source leaf and increased ABA concentration in the leaves, suggesting that the leaves may be the source of ABA present in the seeds (Hein *et al*., 1984).

Photosynthesis is probably the most important metabolic event on earth and is certainly an important process to understand in order to maximize potato productivity (Dean, 1994). It is not the absolute rate of photosynthesis that is important, but rather the relationship between photosynthesis and respiration, identified as the photosynthetic rate. Selection of cultivars with high net photosynthetic rates should result in higher yield if all other factors are equal (Dwelle,

1985). The cultivar CIP-388453-3(A) showed higher rates of leaf net photosynthesis compared to the other tested cultivars. In a field trials Dwelle *et al*. (1981b) screened 17 potato clones and found that clone A6948-4 showed a significantly greater gross photosynthetic rate than the others. The observed genotype differences in relation to photosynthetic efficiency could be a major factor explaining the variation in growth rate and total biomass production. The strong positive association between leaf net photosynthesis and total biomass yield (r = 0.95*) substantiate the postulation. Wilson & Cooper (1970) also observed a positive correlation between shoot dry matter yield and photosynthetic capacity for genotypes of *Lolium perenne*. On the other hand, Werf (1996) reported that although there is much variation among species and genotypes in the rate of photosynthesis per unit leaf area, this variation hardly explains the difference in growth rate between species at similar growth stages. Generally, higher crop yield may not be associated with a higher photosynthetic capacity according to Hay & Walker (1989) because so many canopy characteristics affect productivity.

Fruiting plants showed higher net photosynthetic rates than the flowering and debudded treatments. This may partly be attributed to an increase in assimilate demand. The requirements of the sink organs for photoassimilates regulate the rate of photosynthesis (Ho, 1992). Numerous reports on various crops have shown that increased sink demand results in increased source output (net CO_2 fixation); and decreased sink demand decreased source output (Geiger, 1976; Hall & Milthorpe, 1978; Peet & Kramer, 1980). Pammenter *et al*. (1993) also suggested that low sink demand causes a build-up of assimilates in source leaves and this, in turn, decrease the rate of photosynthesis. A reduced rate of photosynthesis as a consequence of carbohydrate accumulation in leaves was reported in wheat (Azcon-Bieto, 1983) and peanut (Bagnall *et al*., 1988). The observed lower net photosynthetic rate of flowering plants compared to fruiting plants revealed that the growth rate of berries affects the demand for assimilate. Ho (1984) from

his study on the priority of assimilate partitioning in tomato, reported that depending on the availability of assimilates the weaker sinks may or may not receive sufficient assimilates. An initiating inflorescence of tomato is a weaker sink than the shoot apex or roots (Ho *et al*., 1989). The strength of fruit to attract assimilates depends strongly on the developmental stage of the fruit (Heuvelink & Marcelis, 1989; De Koning, 1994).

The photosynthetic efficiency of the leaves of fruiting and non-fruiting plants is regulated by current demand for assimilates and regulatory mechanisms such as hormonal influence, and assimilate concentration (Lenz 1979). Since fruit has relatively high concentrations of phytohormones (Luckwill, 1975; Nitsch, 1970), it has been suggested that hormones deriving from the fruit regulate photosynthesis by directly activating ribulose diphosphate carboxylase (Wareing, 1968). Some experiments have shown that auxin, cytokinin and GA can stimulate the rate of photosynthesis. GA enhanced the activity of ribulose diphosphate carboxylase in leaves (Treharne & Stoddart, 1970; Huber & Sankhla, 1973). Tamas *et al*. (1972) reported that IAA increased photosynthesis of chloroplast through enhancing photophosphorylation. Furthermore, Hoad *et al*. (1977) reported that a change in GA and cytokinin level in grape was observed in response to fruit removal, and ultimately the rate of photosynthesis was altered.

Dry matter production and distribution are crucial processes in determining crop productivity. Cultivars differed with respect to total dry matter production and in the amount allocated to the developing fruit. The cultivar CIP-388453-3(A) produced higher total biomass yield while Al-624 produced the least. A strong correlation between total dry matter yield and net photosynthesis ($r = 0.95^*$) was exhibited, indicating that the variation in photosynthetic efficiency among cultivars substantially contributed to total biomass yield differences. Other researchers also reported the existence of cultivar differences with respect to photosynthetic

efficiency and dry matter production (Hammes & De Jager 1990; Gawronska *et al.,* 1990). Analysing the differences among cultivars with respect to dry matter allocation to the different organs indicated that cultivar Al-624 is less efficient in allocating dry matter to the tubers. The cultivar allotted about 37% of the total dry matter to the leaves and 9.5% to the developing berries and this could be the reason for reduced tuber dry mass. Meyling & Bodlaender (1981) reported that intervarietial differences in tuber yield of the four late cultivars were due largely to differences in the distribution of dry matter. On the other hand, Rijtema & Endrodi (1970) observed a linear relationship between total dry matter, and tuber dry matter and only small differences were observed between cultivars.

The development of berries reduced the partitioning of assimilates to the leaves, stems and roots. Since berries are strong sinks, the reduction may be attributed, at least in part, to the higher assimilate demand for their growth and development. Bartholdi (1940) reported reduced vegetative growth due to flowering and fruiting in potato. Starck *et al*. (1979) observed an increased dry mass of tomato stems and leaves in response to deflowering. Cockshull (1982) reported that the terminal inflorescence buds of C*hrysanthemum morifolium* are stronger sinks for assimilates, and removal of the terminal buds increased the diversion of assimilates to the vegetative organs, particularly to the leaves and roots. Investigating the effect of defoliation and debudding on the root growth of *Taraxacum officinale*, Letchamo & Gosselin (1995) obtained a higher root biomass from debudded than from flowering plants, indicating that flowering has a depressing effect on root growth. The inhibitory effects of reproductive growth on vegetative growth have been reported in tomato (Heuvelink, 1997), apple (Schupp *et al.,* 1992) and chestnut (Famiani *et al*., 2000). Furthermore, the results of many studies on the movement of ^{14}C-assimilates from leaves treated with $^{14}CO_2$ have indicated the capacity of reproductive parts to act

as strong sinks and depress vegetative growth (MacRae & Redgwell, 1990; Eckstein *et al*., 1995; Cruz-Aguado *et al*., 2001).

There is evidence indicating that after fertilization the developing seed and fruit structures are strong sinks and gain priority over vegetative organs in the partitioning of assimilates (Ho, 1988; Ho *et al.*, 1989). This dominance may be mediated by phytohormones as developing seeds and fruit are rich sources of several plant hormones including cytokinins, IAA, ABA and GA_3, although their absolute concentration varies from tissue to tissue within the fruit and is influenced by fruit growth stage (Hedden & Hoad, 1985; Brenner, 1987). Morris (1996) hypothesized that hormones produced by the developing seeds or other fruit parts are exported to other parts of the plants where they induce physiological changes. In soybean, removing the growing pods reduces the level of IAA-esters moving to the source leaf and causes ABA to accumulate in the leaves, suggesting that these may be the source of ABA present in seeds (Hein *et al*., 1984). Brenner (1987) reported IAA stimulates the opening of stomata and IAA protects stomatal closure induced by ABA (Mansfield, 1987). It is believed that developing seeds and fruit can act as a source of auxin for the leaves and sink for leaf produced ABA and thereby regulate assimilate production by promoting CO_2 exchange (Brenner, 1987).

Debudded plants produced a higher aboveground biomass than flowering and fruiting plants. This is due to the production of more lateral branches and expanded leaves in response to removing the flower buds. Salisbury & Ross (1992) reported the existence of apical dominance in the stem of most plant species and pinching off the terminal buds favours the growth of lateral buds and thereby increases branching.

The differences in the growing period of the cultivars may have contributed to the differences in total biomass yield. The observed positive correlation between days elapsed to maturity and total biomass yield (r = 0.84) and maturity period and tuber dry mass (r = 0.99**) support the hypothesis. Iwama *et al.* (1983) also reported that increasing the growing period of potato increased the dry mass of the leaves, stems and roots. Biomass production depends upon leaf canopy size and the duration over the growing season to intercept radiant energy (Van der Zaag, 1984). Allen & Scott (1980) reported that earliness in potato is accompanied by a lower yield. Similarly, Almekinders (1991) reported that the earlier maturing potato variety, Atzimba, produced the least biomass.

Fruit development accelerated the onset of senescence that could be attributed to a competition for nutrients among vegetative and reproductive organs. Developing flowers and fruit are strong sinks for sugar and amino acids and accelerate senescence due to a corresponding decrease in the amounts present in the leaves, according to Salisbury & Ross (1992). They also noted that reproductive organs may produce substances that are transported to vegetative tissue, where they promote senescence.

8.6 CONCLUSION

This study provided evidence that there are cultivar differences with respect to stomatal conductance, rates of leaf transpiration and net photosynthesis. Cultivars also exhibited differences in total biomass yield and allocation among plant organs. Compared to debudded and flowering plants, plants with berries exhibited a higher stomatal conductance and enhanced rate of leaf transpiration that may increase crop water demand and may limit its productivity under water deficit conditions. Although fruit development increased

photosynthetic efficiency, without affecting total dry matter yield, it accelerated plant maturity and decreased the partitioning of assimilates to the leaves, stems and tubers.

CHAPTER 9

GROWTH AND PRODUCTIVITY OF POTATO AS INFLUENCED BY CULTIVAR AND REPRODUCTIVE GROWTH: II. GROWTH ANALYSIS, TUBER YIELD AND QUALITY

9.1 ABSTRACT

A field experiment was conducted under sub-humid tropical conditions in Ethiopia using determinate cultivars Al-624, Al-436, CIP-388453-3(A) and CIP-388453-3(B) to study the effect of flowering and berry set on the growth, tuber yield, and quality of potato. Three treatments viz. debudded, flowering, and fruiting plants were compared and standard growth analysis techniques were applied to study the growth pattern. Fruiting plants exhibited reduced leaf area index, tuber growth rates, and partitioning coefficient, but had higher crop growth rates and net assimilation rates. Fruit development reduced total and marketable tuber mass and tuber number without affecting the unmarketable component. Cultivars varied with respect to tuber yield, tuber number, size distribution, specific gravity, dry matter content, and nutrient composition. Fruiting reduced tuber specific gravity and dry matter content while increasing P, K, Mg, Fe, and Mn content of the tubers. Reproductive growth did not affect tuber Ca, S, Cu, and Zn concentrations. The field experiment demonstrated that reproductive growth restricts vegetative growth and reduces tuber yield and dry matter content of potato.

Keywords: Berry set, dry matter; Ethiopia; growth analysis; tuber quality; tuber yield; specific gravity

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9.2 INTRODUCTION

In most herbaceous annual plants, vegetative growth is terminated by reproductive growth. Developing flowers and fruit are strong sinks for mineral nutrients, sugar and amino acids, and there is a corresponding decrease in the amounts available for the growth of other plant parts (Salisbury & Ross, 1992). Moreover, during the reproductive phase, leaves, stems, and other vegetative parts compete for current assimilates with the developing fruit (Eckstein *et al.*, 1995; Heuvelink, 1997; Famiani *et al.*, 2000) and sometimes previously accumulated carbon and minerals are mobilized and redistributed (Gardner *et al.*, 1985). The distribution of assimilates within the plant is primarily regulated by the sink strength of sink organs (Ho *et al.*, 1989; Marcelis, 1996). Studies in various crops showed that growing fruit is a strong sink and suppresses the growth of vegetative organs (Cockshull *et al.*, 1992; Eckstein *et al.*, 1995; Letchamo & Gosselin, 1995; Heuvelink, 1997).

Albeit the relationship is not well understood, shoot and tuber growth of potato are often considered competing processes (Almekinders & Struik, 1996). The inflorescence as a sink in potato plants has not received adequate research attention and growers view flowers and berries as a minor nuisance. Results with other root crops showed that reproductive growth restricts the development of underground storage organs such as in sugar beet (Wood & Scott, 1975), onion (Khan & Asif, 1981) and Jerusalem artichoke (Rice *et al.*, 1990). However, detailed work has not been done regarding the effect of reproductive growth on potato tuber growth, and results are conflicting. It has been reported that flower formation and berry set have a depressing effect on tuber growth (ProundFoot, 1965; Jansky & Thompson, 1990). On the contrary, Haile-Micheal (1973) observed no consistent relationship between reproductive growth and tuber growth. Tsegaw & Zelleke (2002) showed that reproductive growth restricted vegetative growth and

reduced tuber yield and quality of potato. This finding called for a more detailed investigation of how reproductive growth affects growth, dry matter production and allocation, tuber quality and nutrient composition. This chapter reports on the effect of cultivar and reproductive growth on the growth, yield, quality and nutrient composition of potato tubers.

9.3 MATERIALS AND METHODS

9.3.1 Experimental site description

Detail of the experimental site is described in Chapter 8.

9.3.2 Cultivars

The description of the cultivars is presented in Chapter 8.

9.3.3 General field procedure

The general field procedure is described in Chapter 8.

9.3.4 Treatments

The treatments that were applied are presented in Chapter 8.

9.3.5 Data recorded

Growth analysis

Every 14 days three plants were sampled from each plot and separated into leaves, stems, tubers, and roots and stolons. Green leaf area was measured with a portable CI-202 leaf area meter

(CID Inc., Vancouver, Washington State, USA). Plant tissues were oven dried at 72 °C to a constant mass. The following standard growth analysis parameters were calculated:

$LAI = [(L_{A2} + L_{A1})/2] * (1/G_A)$ (Gardner *et al.*, 1985)

$CGR = 1/G_A * (W_2 - W_1) / (t_2 - t_1)$ (Gardner *et al.*, 1985)

$TGR = 1/G_A * (T_2 - T_1) / (t_2 - t_1)$ (Manrique, 1989)

$FGR = 1/G_A * (F_2 - F_1) / (t_2 - t_1)$

$RGR = ((\ln W_2 - \ln W_1) / (t_2 - t_1))*1000$ (Gardner *et al.*, 1985)

$NAR = [(W_2 - W_1) / (t_2 - t_1)] * (\ln L_{A2} - \ln L_{A1})/ (L_{A2} - L_{A1})]$ (Gardner *et al.*, 1985)

$PC = TGR / CGR$ (Duncan *et al.*, 1978)

Where:

LAI is leaf area index; L_{A2} and L_{A1} are leaf area at time 2 (t_2) and time 1 (t_1), respectively; G_A ground area covered by the crop; CGR is crop growth rate expressed in g m^{-2} day^{-1}, W_2 and W_1 are total crop dry mass (g) at t_2 and t_1; TGR is tuber growth rate expressed in g m^{-2} day^{-1}; T_2 and T_1 are tuber dry mass (g) at t_2 and t_1; FGR is fruit growth rate expressed in g m^{-2} day^{-1}; F_2 and F_1 are fruit dry mass (g) at t_2 and t_1; RGR is relative growth rate expressed in mg g^{-1} day^{-1}; NAR is net assimilation rate expressed in g m^{-2} day^{-1}; PC is partitioning coefficient.

Tuber yield and yield components

Tubers fresh mass and tuber numbers represent the average of 15 plants sampled per a sub-plot. Tubers weighing less than 50 g were considered unmarketable.

Quality assessment

At harvest a representative tuber sample from each plot was taken and washed. Tuber specific gravity was determined by weighing in air and under water (Murphy & Goven, 1959). To determine dry matter content of the tubers the samples were chopped and dried at a temperature of 60 ºC for 15h and followed by 105 ºC for 3h. Tuber dry matter content is the ratio between dry and fresh mass expressed as a percentage.

Samples dried at 60 ºC were analysed for total nitrogen (Macro-Kjeldahl method, AOAC, 1984), and tuber crude protein content was calculated by multiplying total nitrogen by a conversion factor of 6.25 (Van Gelder, 1981). Following wet-ash digestion, phosphorus was determined by colorimetry, potassium by flame photometer, sulphur by turbidimetry, and calcium, magnesium, iron, copper, manganese and zinc by atomic absorption.

9.3.6 Statistical analysis

The analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C, 1991). Means were compared using least significant differences (LSD) test at 5% probability level. Correlations between parameters were computed when applicable. Trends in different growth parameters were analysed by linear regression, using Microsoft Excel 2000.

9.4 RESULTS

For most of the growth parameters considered in the growth analysis there were no differences among the cultivars. Flowering and fruit set influenced most of the growth parameters. During the first harvest period (0-2 weeks), reproductive growth did not influence leaf area index (Figure 9.1). However, during the subsequent sampling periods debudded plants showed consistently higher leaf area indices than plants allowed to flower or set berries.

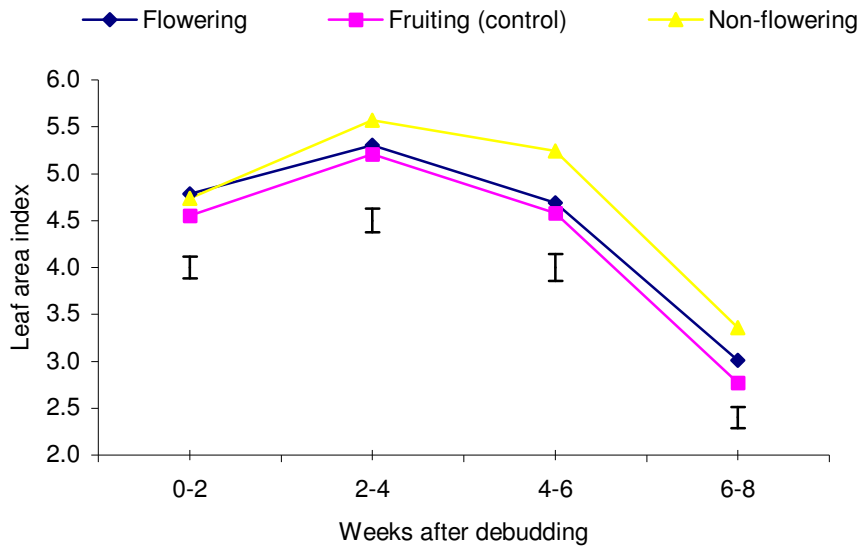

Figure 9.1 The effect of flowering and berry set on leaf area index of potato. The vertical bars represent least significant differences at P < 0.05

The relative growth rate decreased linearly over the eight-week sampling period for all three treatments (Figure 9.2). During the first sampling period, debudded plants exhibited a higher relative growth rate (21 mg g^{-1} day^{-1}) than flowering (19 mg g^{-1} day^{-1}) and fruiting (18.0 mg g^{-1} day^{-1}) plants. For the third sampling period, fruiting plants had a higher relative growth rate than other treatments, while during the second and fourth observation periods no differences occurred.

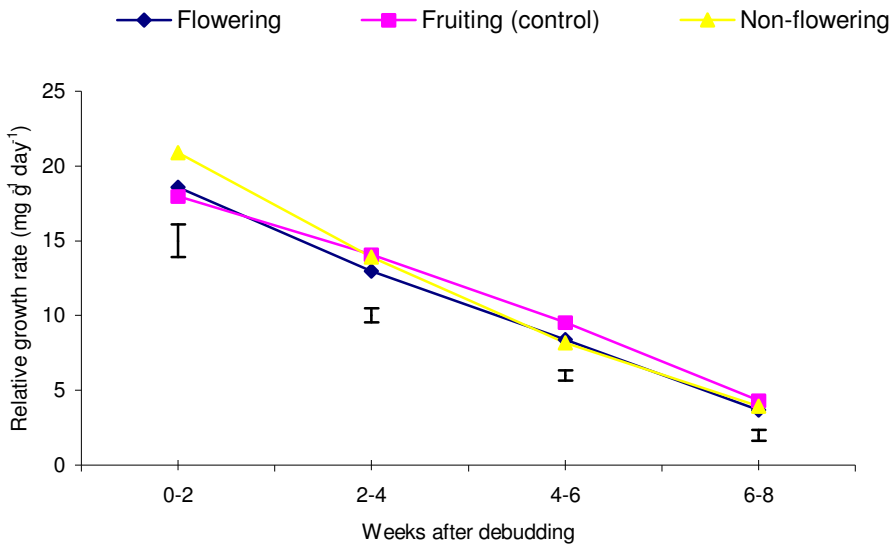

Figure 9.2 Relative growth rate of potato as affected by flowering and berry set. The vertical bars represent least significant differences at P < 0.05

The net assimilation rate declined from about 3 g m^{-2} day^{-1} to nearly 1.6 g m^{-2} day^{-1} towards maturity (Figure 9.3). During the first sampling period (0-2 weeks), debudded plants had the highest net assimilation rate (3.2 g m^{-2} day^{-1}) and flowering plants the lowest (2.6 g m^{-2} day^{-1}). During the second sampling period, fruiting plants showed a higher net assimilation rate than flowering plants while the debudded plants were intermediate. During the subsequent samplings, fruiting plants exhibited a higher net assimilation rate than flowering and debudded plants.

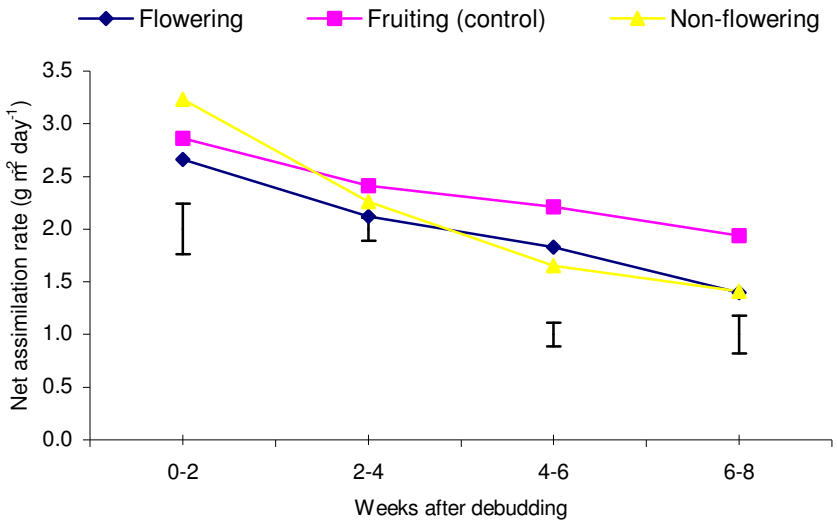

Figure 9.3 Net assimilation rate of potato as affected by flower and berry production. The vertical bars represent least significant differences at P < 0.05

Crop growth rate declined sharply from over 12 g m^{-2} day^{-1} (during 0-2 weeks) to less than 5 g m^{-2} day^{-1}during the final sampling period (Figure 9.4). From the time of debudding up to the second week, debudded plants exhibited a higher crop growth rate than flowering and fruiting plants. During the two to four week period, debudded and fruiting plants had higher crop growth rates. During the third sampling period fruiting plants showed higher crop growth rates than the other treatments. Towards maturity comparable crop growth rate of about 4.4 g m^{-2} day^{-1} was recorded for all three treatments.

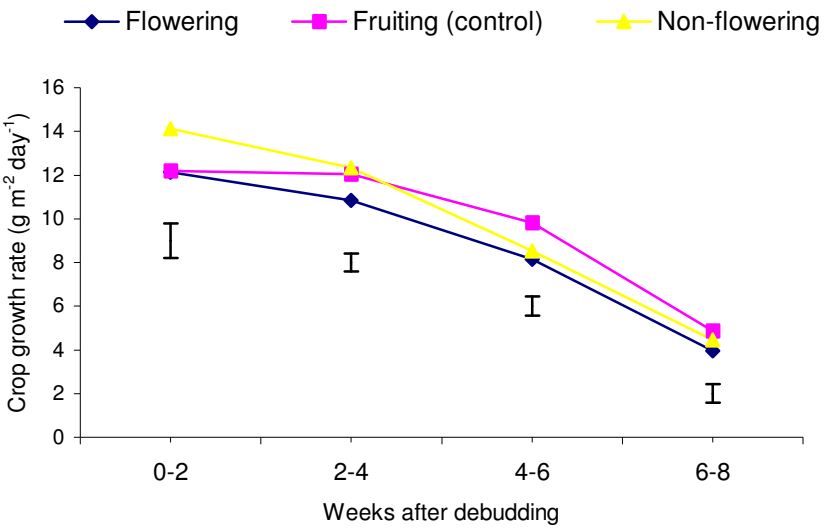

Figure 9.4 The effect of flowering and berry set on potato crop growth rate. The vertical bars represent least significant differences at P < 0.05

Fruit growth rate pooled over cultivars is presented in Figure 9.5. The fruit growth rate increased progressively from 1.14 g m^{-2} day^{-1} (0-2 weeks) to a peak of 1.7 g m^{-2} day^{-1} during the third sampling period (4-6 weeks), and declined sharply towards maturity.

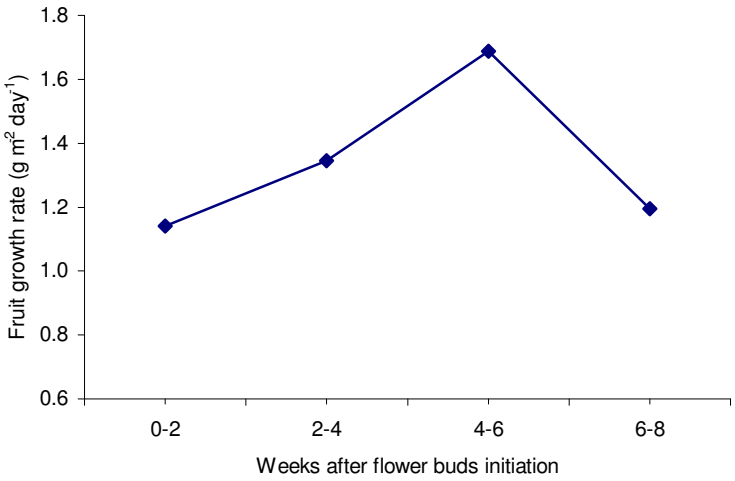

Figure 9.5 The growth rate of potato berry. Mean of four cultivars

Peak tuber growth rates were recorded two to four weeks after flower bud removal and declined afterwards (Figure 9.6). At all sampling periods, the debudded plants demonstrated a higher tuber growth rate than fruiting plants.

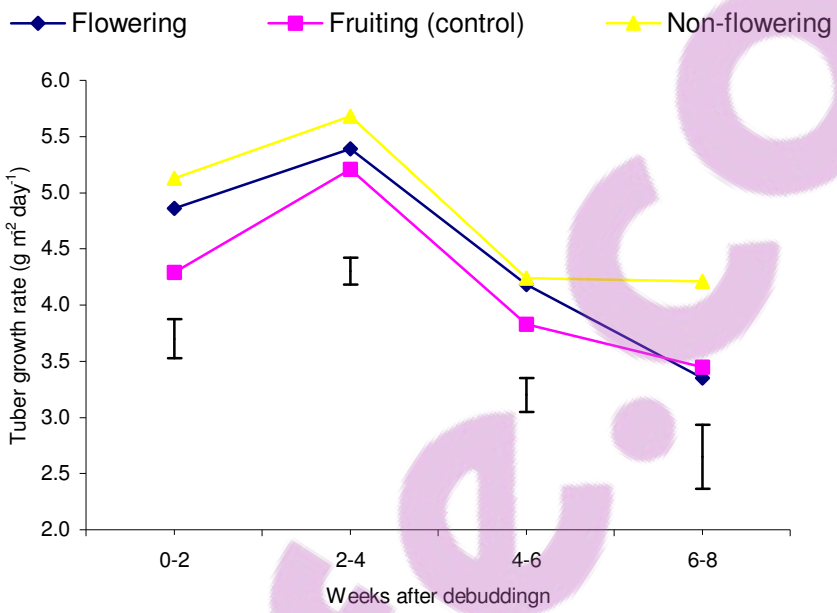

Figure 9.6 The effect of flowering and berry set on tuber growth rate of potato. The vertical bars represent least significant differences at P < 0.05

The partitioning coefficient illustrated in Figure 9.7 indicates the ratio of tuber growth rate to crop growth rate. Except for the second harvesting phase (2-4 weeks after debudding), fruiting plants exhibited a lower partitioning coefficient than non-flowering plants.

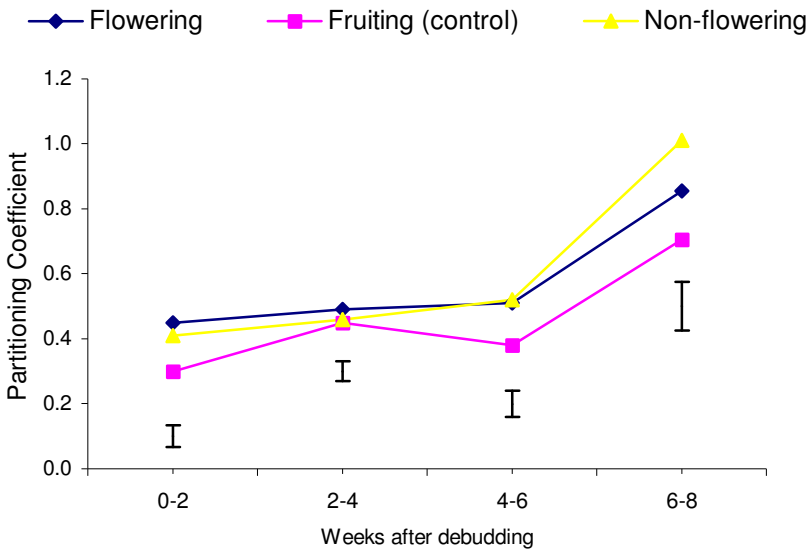

Figure 9.7 Partitioning coefficient of potato as affected by flower and berry development. The vertical bars represent least significant differences at P < 0.05

Differences between cultivars in total, marketable, and unmarketable tuber yield are presented in Table 9.1. Cultivar CIP-388453-3(A) produced the higher total tuber yield (991 g hill^{-1}), followed by Al-436 (849 g hill^{-1}), CIP-388453-3(B) (711 g hill^{-1}), and Al-624 (567 g hill^{-1}). Al-624 had a much smaller proportion of unmarketable (smaller tubers) than the other three cultivars. Cultivars CIP-388453-3(B), Al-436 and CIP-388453-3(A) produced a higher proportion of small tubers than Al-624. A significant difference was observed among cultivars with respect to total number of tubers (Table 9.1). CIP-388453-3(B) and A-436 produced a total of about 15 tubers, followed by CIP-388453-3(A) and Al-624 producing 12 and 5 tubers per hill, respectively. Fruit development decreased the productivity by reducing both tuber size and number. Without affecting the unmarketable component, fruit development reduced the total and marketable tuber yield by about 19 and 22%, respectively, as compared to the other two treatments. Similarly, without affecting the unmarketable component, fruit development decreased the total and marketable number of tubers.

Table 9.1 Total, marketable and unmarketable tuber yield and number of potato as influenced by cultivar and flowering and fruit set

	Tuber yield (g hill^{-1})			Tuber number (hill^{-1})		
Main effect	Total	Marketable	Unmarketable	Total	Marketable	Unmarketable
Cultivar						
CIP-388453-3(A)	991.0a	837.3a	153.7a	11.6b	6.4a	5.2b
A-624	566.7d	517.6c	49.1b	5.4c	3.5b	1.9c
Al-436	849.2b	672.8b	176.4a	14.0a	7.8a	6.2b
CIP-388453-3(B)	711.5c	525.2c	186.3a	15.3a	5.9ab	9.4a
SEM	21.52	15.64	10.78	0.45	0.22	0.52
Treatment						
Non-flowering	844.3a	696.6a	147.7a	12.2a	6.2a	6.0a
Flowering	822.9a	678.4a	144.5a	11.8a	6.2a	5.6a
Fruiting (control)	671.6b	539.7b	131.9a	10.7b	5.3b	5.4a
SEM	14.92	13.91	6.20	0.16	0.23	0.24

SEM: Stand error of the mean.

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

The cultivars differed in tuber dry matter content as well as specific gravity (Table 9.2). CIP-388453-3(A) and CIP-388453-3(B) produced tubers containing approximately 22% dry matter which is higher than the tuber dry matter content of Al-436 and Al-624 (19%). Cultivars in decreasing order of tuber specific gravity are CIP-388453-3(A) (1.090 g cm^{-3}), CIP-388453-3(B) (1.085 g cm^{-3}), Al-436 (1.076 g cm^{-3}), and Al-624 (1.070 g cm^{-3}). The presence of berries reduced tuber dry matter content as well as specific gravity. Fruit development reduced tuber dry matter content by about 3.3% compared to non-flowering plants. Tubers of the non-flowering and flowering plants showed higher specific gravity (1.081 g cm^{-3}) than the fruiting ones (1.078 g cm^{-3}).

Table 9.2 The effect of cultivar and reproductive growth on dry matter content, specific gravity, crude protein content, and macroelement content of potato tubers

Main effect	Dry matter content (%)	Specific gravity (g cm^{-3})	Crude protein (%)	P (%)	K (%)	Ca (%)	S (%)	Mg (%)
Cultivar								
CIP-388453-3(A)	22.8a	1.090a	5.6d	0.26b	2.25c	0.060b	0.08d	0.132b
A-624	18.6b	1.070b	10.1a	0.34a	3.00a	0.072a	0.50a	0.159a
Al-436	19.8b	1.076ab	7.4b	0.26b	2.42b	0.054b	0.15c	0.132b
CIP-388453-3(B)	21.8a	1.085ab	6.8c	0.28ab	2.27c	0.059b	0.22b	0.128b
SEM	0.39	0.002	0.04	0.002	0.02	0.002	0.007	0.001
Treatment								
Non-flowering	21.0a	1.081a	7.4b	0.28b	2.44b	0.060a	0.22a	0.136b
Flowering	20.9a	1.081a	7.3b	0.28b	2.47b	0.063a	0.25a	0.137b
Fruiting (control)	20.3b	1.078b	7.8a	0.29a	2.53a	0.061a	0.24a	0.141a
SEM	0.11	0.001	0.03	0.001	0.007	0.001	0.12	0.001

SEM: Stand error of the mean.

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

The cultivars differed with respect to tuber crude protein content and the concentration of macronutrients as indicated in Table 9.2. Cultivar Al-624 produced tubers with a higher crude protein content (10%), followed by Al-436 (7.4%), CIP-388453-3(B) (6.8%), and CIP-388453-3(A) (5.6%). Cultivar Al-624 also produced tubers with higher phosphorus, potassium, calcium, sulphur, and magnesium contents compared to the other cultivars. Interestingly, fruit development increased tuber crude protein content and phosphorus, potassium, and magnesium content without affecting calcium and sulphur (Table 9.2). Fruiting plants produced tubers containing higher crude protein, phosphorus and potassium content than tubers from the non-flowering and flowering treatments. The three treatments had comparable tuber calcium (0.06%) and sulphur (0.24%) contents.

The mean copper content of the tubers were 20 ppm for Al-624, CIP-388453-3(A) and CIP-388453-3(B) which was higher than in the case of cultivar Al-436 (18 ppm). Cultivars Al-624

and CIP-388453-3(B) had the highest tuber zinc content. All of the cultivars produced tubers with comparable iron (56 ppm) and manganese (3.8 ppm) contents. Tubers of fruiting plants contained more iron (61 ppm) than tubers of non-flowering and flowering plants (54 ppm) (Table 9.3). A higher tuber manganese concentration was observed in fruiting plants (4.9 ppm). Reproductive growth did not affect tuber copper and zinc concentrations.

Table 9.3 The effect of cultivar and reproductive growth on tuber microelement content

Main effect	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
Cultivar				
CIP-388453-3(A)	20.17a	54.47a	3.96a	13.83c
A-624	21.67a	59.17a	4.33a	20.83a
Al-436	18.00b	59.67a	3.00a	13.17c
CIP-388453-3(B)	20.00a	51.33a	3.83a	18.61ab
SEM	0.36	3.08	0.42	1.40
Treatment				
Non-flowering	19.25a	52.75b	3.75ab	14.75a
Flowering	20.25a	54.37b	2.74b	19.08a
Fruiting (control)	20.37a	61.13a	4.87a	16.00a
SEM	0.38	1.38	0.44	1.28

SEM: Stand error of the mean.

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

The macro- and microelement composition of potato berries is presented in Table 9.4. Mean berry nitrogen content was 2.2%, phosphorus 0.4%, potassium 3.7%, calcium 0.2%, sulphur 0.5%, magnesium 0.3%, copper 24 ppm, iron 94.2 ppm, manganese 6.8 ppm, and zinc 29 ppm. The berries contained higher concentrations of macro and micronutrients than the tubers.

Table 9.4 The concentrations of macro and micronutrients in the berries of four potato cultivars

Cultivar	N (%)	P (%)	K (%)	Ca (%)	S (%)	Mg (%)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Zn (ppm)
CIP-388453-3(A)	2.10	0.40	3.81	0.20	0.40	0.24	23.7	93.7	7.01	23.67
A-624	2.23	0.47	3.93	0.19	0.42	0.29	25.0	96.3	7.33	33.33
Al-436	2.18	0.41	3.73	0.18	0.44	0.26	24.0	94.2	6.33	27.33
CIP-388453-3(B)	2.23	0.40	3.45	0.20	0.67	0.28	23.3	92.7	6.67	32.00
Mean	2.18	0.42	3.73	0.19	0.48	0.27	24.0	94.2	6.8	29.08

9.5 DISCUSSION

Depending on the strength of the sinks, potato plants allocate assimilates to the developing fruit, tubers and other vegetative structures. Under conditions of assimilate limitation competition among sink organs is imperative. Treatments that increase the partitioning of assimilates to the tubers and/or reduce utilization by other organs are likely to favour tuber growth and increase yield.

Debudded and flowering plants had higher leaf area indices which is attributed to the development of more lateral branches with larger leaves in response to apical bud and flower removal. Chatfield *et al.* (2000) reported that shoot apical meristem maintains its role as the primary site of growth by inhibiting the growth of axillary meristems. `

Fruiting plants exhibited higher crop growth rates compared to the flowering and non-flowering plants. The higher crop growth rates may be attributed to the increased photosynthetic efficiency (Chapter 8) and enhanced net assimilation rates. In a tomato, Starck *et al.* (1979) observed increased net photosynthesis and net assimilation rates in fruiting plants compared to deflorated plants.

Fruit development reduced partitioning of assimilates to the tubers and thereby suppressed tuber growth. This may probably be attributed to the strong assimilate attraction power of developing fruit. There is evidence that the developing seed and fruit are strong sinks which have priority over vegetative organs in the partitioning of assimilates (Ho, 1988; Ho *et al.*, 1989). This dominance is believed to be mediated by phytohormones, because developing seeds and fruit are rich sources of several plant hormones, including cytokinins, IAA, ABA and GA_3 (Hedden & Hoad, 1985; Brenner, 1987).

The efficiency of dry matter accumulation by the tubers was assessed by the partitioning coefficient. Berry development reduced the partitioning coefficient by about 24% as compared to debudded and flowering plants. The partitioning coefficients increased progressively over time indicating that an increasing fraction of available assimilates were allocated to the tuber growth as the crop matured.

In the fruiting plants, the proportion of dry matter partitioned to the berries varied from 5 to about 9% of the total carbon fixed. The maximum fruit growth rate was observed 4-6 weeks after flower bud initiation. A few days after pollination, potato berries start active growth and attain full development after six weeks, according to Sadik (1983).

The cultivars exhibited differences with respect to tuber yielding potential. This could be attributed to variation in days to tuber initiation, rate of photosynthesis, efficiency of assimilate partitioning to the tubers (bulking rate) and maturity period. The strong positive correlation of tuber yield with leaf net photosynthesis ($r = 0.97^{**}$), and days to maturity ($r = 0.84$) supports the speculation. Hammes & De Jager (1990) and Gawronska *et al*. (1990) reported the existence of varietial differences with respect to the rate of net photosynthesis and dry matter production.

Berry development reduced total tuber yield. This indicates that reproductive development had a depressing effect on tuber growth, which may partly be due to competition for assimilates. The strong negative correlation observed between total tuber yield and berry yield (r = -95*) and total tuber yield and berry number (r = -0.99**) signified that assimilate allocation to the tubers was to a large extent determined by the number and size of the berries. Fruit number and size determined biomass allocation in pepper (Nielsen & Veierskov, 1988) and kiwifruit (Richardson & MacAneny, 1990). Tsegaw & Zelleke (2002) conducted an experiment with the same potato cultivars and at the same location in Ethiopia and found that berry development reduced total tuber yield by about 17% compared to the non-flowering plants. ProundFoot (1965) and Jansky & Thompson (1990) also reported that berry development reduced tuber yield. However, Haile-Micheal (1973) reported no consistent relationship between reproductive growth and tuber yield in potato. Results of studies on other crops have also indicated that flower and fruit compete for assimilates and thereby depress the development of underground storage organs such as in sugar beet (Wood & Scott, 1975), onion (Khan & Asif, 1981) and Jerusalem artichoke (Rice *et al*., 1990).

Variation in tuber number among the tested cultivars indicated that there were considerable differences with respect to number of tubers initiated in the course of development. Except for cultivar CIP-388453-3(A), tuber numbers increased in response to debudding, indicating tuber initiation after blooming.

The increase in the proportion of marketable tubers as a consequence of suppressing berry development may be explained on the bases of absence of competition for assimilates between developing fruits and tubers. It is speculated that in the absence of reproductive parts, presumably since developing tubers are the predominant sinks, a large amount of dry matter is

diverted to the tubers which would otherwise be utilized for reproductive growth. As a result, most of the initiated tubers increased in size. The increase in dry matter content of tubers also substantially contributed for tuber yield improvement as indicated on a strong association between them (r = 0.73)

The variation in specific gravity and dry matter content among cultivars can be attributed to the variation in efficiency of diverting of more dry matter to the tubers. Dean (1994) indicated that although tuber dry matter content is influenced by tuber size, environmental conditions and cultural practices, tuber dry matter content appear to be genetically controlled. Lana *et al.* (1970) and Kushman & Haynes (1971) reported that variation in tuber specific gravity could be due to variation in tissue specific gravity and amount of intercellular space in the tubers. The variation in specific gravity could be due to differences in starch grain size, according to Sharma and Thompson (1956). The highly significant positive correlation (r = 0.99**) observed between specific gravity and percent dry matter indicates that specific gravity is a good indicator of tuber dry matter content. Porter *et al.* (1964) and Fitzpatrick *et al*. (1964) reported a positive correlation between specific gravity and percent dry matter. On the contrary, however, Wilson & Mlindsay (1969) reported a hyperbolic relationship between them.

Fruit development decreased tuber specific gravity and dry matter content, which may be explained on the basis of competition for assimilates between developing berries and tubers. In the absence of reproductive parts, more assimilates are presumably diverted and accumulated in the tubers. The observed negative relations between fruit yield and tuber dry matter content (r = -0.82) and fruit yield and specific gravity (r = -0.83) support the speculation. Tsegaw & Zelleke (2002) also reported that reproductive growth reduced tuber specific gravity and dry matter content..

Potato berries contained higher macro- and micronutrient concentrations than the tubers, indicating that they are strong sinks for mineral elements. Cultivars differed in tuber macro- and micronutrient concentrations. Cultivar Al-624 produced tubers containing higher concentrations of most major and trace elements than the other cultivars. Fruit development increased the concentration of tuber N, P, K, Mg, Fe, and Mn without affecting Ca, S, and Cu concentrations. Fruiting plants exhibited higher tuber nutrient content and rate of transpiration (Chapter 8). This association strengthens the hypothesis that an increased rate of transpiration enhances the rate of mineral uptake. Salisbury & Ross (1992) reported that growing plants in greenhouses where there is reduced transpiration due to high humidity may cause calcium deficiencies in certain tissues and too rapid transpiration can lead to a toxic build up of certain elements. In the current study, it was found that fruit development reduced tuber yield by reducing both tuber size and number. Hence, the observed lower concentration of macro and micronutrients in relatively larger tubers of the debudded and flowering plants may partly be a consequence of a "dilution effect".

9.6 CONCLUSION

Cultivars differences with respect to tuber fresh mass, tuber number, specific gravity, dry matter content, and nutrient composition were recorded and should be exploited in cultivar development. Fruit development reduced the leaf area index, tuber growth rate and partitioning coefficient while increasing the crop growth rate and net assimilation rate. Fruit development decreased tuber yield as well as dry matter content. Prevention of berry set by potato growers should increase tuber yield and dry matter content. Hence, simple and economical means to control flowering and berry set should be investigated. It was noticed in the trials that in addition to the reported advantages, PBZ also prevented flowering.

Consequently, trials were devised to compare the efficacy of PBZ with other chemicals proven effective as flower-controlling agents, and the results are presented in Chapter 10.

CHAPTER 10

THE EFFECT OF MCPA AND PACLOBUTRAZOL ON FLOWERING, BERRY SET, BIOMASS PRODUCTION, TUBER YIELD AND QUALITY OF POTATO

10.1 ABSTRACT

The effects of MCPA and PBZ on flowering, berry formation, dry matter production and allocation, tuber yield and quality of potato were investigated under greenhouse and field conditions at the Experimental Farm of the University of Pretoria. Both MCPA and PBZ were applied during the early and full bud stages at rates of 0, 5, 10, and 15 mg a.i. plant^{-1} (greenhouse trial) and 0, 250, 500, and 750 g a.i. ha^{-1} (field trial) Regardless of rate and stage of application, MCPA and PBZ prevented flowering and completely inhibited berry set. MCPA did not affect the number, yield, specific gravity and dry matter content of the tubers. Without affecting the number of tubers initiated, PBZ increased tuber yield, specific gravity and dry matter content. MCPA decreased assimilate partitioning to the stems. PBZ treatment at early flower bud stage resulted in a higher tuber yield than spraying during late flower bud stage. PBZ decreased assimilate partitioning to the leaves, stems, and roots while it increased tuber yield. A single foliar spray of MCPA or PBZ at the early flower bud stage at a rate of 250g a.i. ha^{-1} is effective to reduce flower formation and prevent berry set.

Keywords: Berry set, flowering, MCPA, paclobutrazol, potato, quality, yield

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10.2 INTRODUCTION

Flowering in potato occurs in various degrees depending on the species, cultivar and environmental conditions (Sadik, 1983; Lozaya-Saldana, 1992). Its expression is influenced by internal and external factors including source-sink equilibrium, hormonal balance, physiological maturity and photoperiod (Lozaya-Saldana & Miranda-Verlazguez, 1987; Lozaya-Saldana, 1992).

The berry of potato is spherical with a diameter of 1.2 to 1.9 cm, green or purplish green tinged with violet, and contains numerous small seeds (Smith, 1968). Fruit set often does not take place, even when conditions are ideal for flowering (CIP, 1983). This seems to suggest that favourable conditions for flowering are not necessarily optimal for the processes of pollination and fruit development. Flower abscission can occur due to one or more factors such as lack of insect pollinators, poor pollen viability or temperatures too low for pollen germination and fertilization (Sadik, 1983). He also indicated that it may be due to a competition between the berries and tubers for limiting factors.

In most potato growing areas of Ethiopia the majority of the cultivars produce flowers and some of them set berries. Some of the promising elite genotypes produce many berries. The use of true potato seed has been limited to breeding and selection purposes at experimental stations. Previous reports indicated that reproductive development in potato restricts vegetative growth and tuber growth (ProundFoot, 1965; Jansky & Thompson, 1990; Tsegaw & Zelleke, 2002). In Chapter 8 and Chapter 9 it was reported that flowering and berry formation decreased vegetative growth, reduced tuber yield and quality. In addition, potato seed can remain viable in the soil for more than 10 years and be a source of unwanted volunteer plants (Lawson, 1983) that may act as weeds and alternate hosts for the persistence of nematodes, viruses, fungi and bacteria

(Lutman, 1977). Hence, the formation of berries in potato is an undesirable characteristic and there is a need for efficient control measures.

Researchers have tested different chemicals to control berry set. According to Wedgwood (1988), the best control of berry production was achieved with a combination of MCPB and Bentazone applied at the full foliage to flowering stage. Veerman & Van Loon (1993) screened MCPA, ethephone, 2,4D-amine, naphtylacetamide, metoxuron and gibberellic acid in four experiments and reported that MCPA and ethephon reduced berry set when applied at the early flower bud stage. Casual observations in trials reported in Chapter 3, Chapter 4, and Chapter 5 indicated that PBZ inhibited flower formation and berry set in potato. This Chapter reports on the effect of different rates of a single foliar spray of MCPA or PBZ applied at early or full flower bud stage on flowering, berry set, biomass production and partitioning, tuber yield and quality of potato. The objective was to determine if MCPA and PBZ can be used to control flowering and berry set without negatively affecting yield parameters.

10.3 MATERIAL AND METHODS

10.3.1 Greenhouse experiments

Separate greenhouse trials were carried out with MCPA and PBZ at the Experimental Farm of the University of Pretoria from December 2003 to March 2004. Single plant of the potato cultivar BP1 were grown in 5-liter plastic containers using a mixture of sand and coconut coir (50:50 by volume) as a growing medium. In both trials, the treatments were arranged in a two factor (rate and stage of application) factorial combination in a randomised complete block design with three replications.

Both MCPA ((4-chloro-2-methylphenoxy) acetic acid) and PBZ were applied at rates of 0, 5, 10, and 15 mg plant^{-1}; approximately equivalent to 0, 250, 500, and 750 g a.i. ha^{-1}, respectively. A single foliar spray was applied at early flower bud stage when the first flower buds started to emerge, or at full bud stage (8 days after the first application, when flower buds were swollen but before flower opening) as a fine foliar spray using an atomizer. The control plants were treated with distilled water.

During the growing period diurnal temperatures ranged between 17 and 35 ºC, and the average relative humidity was 54%. Plants were fertilized with a standard Hoagland solution and watered regularly to avoid water stress.

10.3.2 Field experiments

Separate field trials were conducted from December 2003 to March 2004 at the Experimental Farm of the University of Pretoria (25^{o} 45‘ S; 28^{o} 16‘ E; altitude 1372 m a s l) using the cultivar BP1. In both trials, treatments were arranged in a two factor (rate and stage of application) factorial combination in a randomised complete block design with three replications. Medium sized, well-sprouted tubers of cultivar BP1 were planted at a spacing of 75 x 30 cm. A row of six plants represented a treatment plot. Plots were arranged continuously without board rows, and the end plots were bordered by two rows of potato plants. Plots were fertilized with 555 kg ha^{-1} of a 2: 3: 2(30) compound fertilizer and irrigated regularly to maintain adequate moisture in the soil.

A single foliar spray of both MCPA and PBZ were applied at rates of 0, 250, 500, and 750 g a.i. ha^{-1}.

The soil of the experimental site is sandy clay with 0.12% total nitrogen, 3 ppm phosphorus, 24 ppm potassium, and a pH (H_20) of 6.9. During the growing period the daily minimum temperatures ranged from 10 to 24 ºC while the maximum was between 15 and 37 ºC. Plants received a total of about 600 mm rainfall and supplemental irrigation was applied whenever necessary to prevent water stress.

10.3.3 Data recorded

Flowers were counted every other day. Flowers numbers represent the total number of open flowers observed per hill. Berry numbers indicate the number of mature berries per hill at harvest.

At the final harvest (eight weeks after the last treatment application) of the greenhouse trial, two pots per treatment were harvested and separated into berries, leaves, stems, tubers, and roots and stolons. In the field trial, two plants per plot were sampled for dry matter partitioning seven weeks after the last treatment application. A week later, the remaining four plants were harvested for yield and quality determination.

Plant tissues samples were oven dried at 72 °C to a constant mass. Dry matter partitioning was determined from the dry mass of individual plant parts as a percentage of total plant dry mass. Tuber specific gravity was determined using the weight in air and weight in water method. For dry matter content tubers were oven dried at a temperature of 72 ºC to a constant mass. Total dry matter content of the tubers was calculated as the ratio between dry and fresh mass expressed as a percentage.

10.3.4 Statistical analysis

The analyses of variance were carried out using MSTAT-C statistical software (MSTAT-C 1991). Means were compared using the least significant differences (LSD) test at the 5% probability level.

10.4 RESULTS

Greenhouse experiments

The effect of MCPA and PBZ on the number of flowers and berries is presented in Table 10.1. Irrespective of the concentration and stage of application, a single foliar spray of MCPA or PBZ prevented flowering and completely inhibited berry set in potato (Fig. 10.1)

Table 10.1. Number of flowers and berries after application of MCPA or PBZ at early or full flower bud stage: Greenhouse trial

Application		MCPA		PBZ	
Stage	Rate (mg plant^{-1})	Number of flowers	Number of berries	Number of flowers	Number of berries
Early bud	0 (control)	44.67a	5.67a	48.9a	6.1a
	5	0.00b	0.00b	0.0b	0.0b
	10	1.33b	0.00b	0.0b	0.0b
	15	0.67b	0.00b	0.0b	0.0b
Full bud	0 (control)	45.67a	6.00a	44.6a	5.4a
	5	2.00b	0.00b	1.30b	0.0b
	10	3.67b	0.00b	0.80b	0.0b
	15	1.00b	0.00b	0.00b	0.0b
	SEM	3.31	0.15	2.05	0.26

SEM: Standard error of the mean.

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

Figure 10.1 Application of MCPA at a rate of 10 mg plant^{-1} (B) and PBZ at a rate of 10 mg plant^{-1} (C) inhibited berry set compared to the control (A)

Table 10.2 shows the effect of MCPA and PBZ treatment on tuber number, yield and quality for the greenhouse trial. MCPA did not affect tuber number, yield, specific gravity and dry matter content. Similarly, PBZ treatment did not affect the number of tubers initiated. Regardless of the stage of application, however, PBZ increased tuber yield, specific gravity and dry matter content. PBZ treatment at a rate of 10 or 15 mg plant^{-1} increased tuber yield by about 31% over the control. Irrespective of the concentrations, PBZ treatment increased specific gravity by about 1.5% and dry matter content by 27%. A strong correlation (r = 0.99**) was observed between tuber specific gravity and dry matter content.

Table 10.2. Tuber number, yield, specific gravity, and dry matter content as affected by rates of MCPA and PBZ applied during early or full flower bud stage: Greenhouse trials

Treatment	MCPA greenhouse experiment				PBZ greenhouse experiment			
	Tuber number (pot^{-1})	Tuber yield ($g\ hill^{-1}$)*	Specific gravity ($g\ cm^{-3}$)	Dry matter (%)	Tuber number (pot^{-1})	Tuber yield ($g\ hill^{-1}$)*	Specific gravity ($g\ cm^{-3}$)	Dry matter (%)
Stage								
Early bud	11.2a	492(94)a	1.043a	12.7a	11.5a	510(92)a	1.050a	14.3a
Full bud	9.3a	485(93)a	1.050a	14.3a	10.8a	506(93)a	1.048a	13.6a
SEM	0.88	17.8	0.003	0.63	0.68	18.6	0.002	0.52
Rate ($mg\ plant^{-1}$)								
0 (control)	11.3a	483(92)a	1.039a	12.0a	9.0a	432(93)b	1.038b	11.6b
5	11.7a	514(94)a	1.046a	13.4a	9.7a	467(93)b	1.052a	14.2a
10	10.5a	494(93)a	1.046a	13.3a	12.6a	584(93)a	1.055a	15.2a
15	7.5a	465(95)a	1.055a	15.3a	13.3a	548(92)a	1.052a	14.8a
SEM	1.25	8.94	0.004	0.89	0.96	24.7	0.003	0.73

* Figures in parenthesis represent the percentage of tubers larger than 50 g.

SEM: Stand error of the mean.

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

Table 10.3 indicates dry matter production and allocation as affected by MCPA and PBZ treatment in the greenhouse. The stage of application did not alter the effect of MCPA on total dry matter production as well as allocation amongst organs. Without affecting the total biomass production and assimilate partitioning to the underground parts, MCPA treatment decreased leaf and stem mass. PBZ treatment during the early flower bud stage resulted in a higher tuber mass than applying during the late flower bud stage. PBZ treatment decreased leaf mass while increasing tuber mass.

Table 10.3. Total biomass production and allocation to the different parts of potato after a single application of MCPA or PBZ: Greenhouse trials

Treatment	Leaf (g)	Stem (g)	Fruit (g)*	Root & stolon (g)	Tuber (g)	Total biomass (g)
MCPA						
Early bud	20.3(17)a	14.1(12)a	11.30(10)a	8.0(7)a	65.2(55)a	118.9a
Full bud	18.6(16)a	12.3(10)b	11.28(9)a	7.4(6)a	69.6(58)a	119.2a
SEM	0.89	0.48	0.13	0.75	2.29	2.46
0 (control)	22.7(19)a	16.5(14)a	11.3(10)a	6.5(5)a	61.2(52)a	118.2a
5 (mg plant^{-1})	18.9(17)ab	12.8(12)b	00.0(0)b	9.0(8)a	67.4(62)a	108.2a
10 (mg plant^{-1})	18.2(17)b	12.0(11)b	00.0(0)b	8.0(7)a	70.2(65)a	108.4a
15 (mg plant^{-1})	18.0(17)b	11.6(11)b	00.0(0)b	7.3(7)a	70.7(66)a	107.6a
SEM	1.26	1.15	0.19	1.05	6.27	6.91
PBZ						
Early bud	20.0(15)a	15.3(12)a	13.5(10)a	7.8(6)a	72.5(56)a	123.1a
Full bud	19.2(16)a	13.3(11)a	13.6(11)a	8.0(7)a	66.5(55)b	126.6a
SEM	0.53	0.77	0.08	0.81	1.60	1.76
0 (control)	23.0(20)a	15.3(13)a	13.6(12)a	7.3(6)a	55.9(49)c	114.9a
5 (mg plant^{-1})	19.2(17)b	13.6(12)a	00.0(0)b	8.6(8)a	68.6(62)b	110.1a
10 (mg plant^{-1})	17.2(15)b	14.8(13)a	00.0(0)b	7.8(7)a	76.9(66)a	116.7a
15 (mg plant^{-1})	18.8(16)b	13.7(12)a	00.0(0)b	7.9(7)a	76.6(65)ab	117.0a
SEM	1.05	1.69	0.12	1.14	6.05	6.31

Figures in parenthesis represent percentage of the total biomass.
* Mean values for the rates of MCPA and PBZ are the average of the three replications.

SEM: Stand error of the mean.

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

Field experiments

The effect of MCPA and PBZ on the number of flowers and berries of potato grown under field conditions is presented in Table 10.4. Irrespective of the concentration and stage of application, a single foliar spray of MCPA or PBZ prevented flowering and completely inhibited berry set.

Table 10.4. Number of flowers and berries after application of MCPA or PBZ at early or full flower bud stage: Field trials

Application		MCPA		PBZ	
Stage	Rate (g ha^{-1})	Number of flowers	Number of berries	Number of flowers	Number of berries
Early bud	0 (control)	53.33a	4.33a	54.7a	5.62a
	250	3.00b	0.00b	1.6b	0.0b
	500	0.53b	0.00b	0.0b	0.0b
	750	0.00b	0.00b	0.0b	0.0b
Full bud	0 (control)	52.33a	5.47a	47.3a	5.91a
	250	1.83b	0.00b	0.0b	0.0b
	500	0.00b	0.00b	2.03b	0.0b
	750	0.00b	0.00b	1.23b	0.0b
	SEM	2.15	0.39	1.80	0.16

SEM: Standard error of the mean.

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

The effect of MCPA and PBZ on yield and quality of potato is indicated in Table 10.5. MCPA did not affect tuber number, yield, specific gravity or dry matter content. Without affecting tuber number, specific gravity and dry matter content, early PBZ treatment resulted in better tuber yield than late application. Applying 500 or 750 g of PBZ ha^{-1} increased tuber yield by about 24%, specific gravity by 1.2%, and dry matter content by 19% over the control. There was a strong correlation (r =0.99**) between specific gravity and dry matter content.

Table 10.5. Tubers number, tuber mass, specific gravity, and dry matter content of potato as affected by rates of MCPA and PBZ applied during early or full flower bud stages: Field trials

Treatment	MCPA field experiment				PBZ Field experiment			
	Tuber number (hill^{-1})	Tuber mass (g hill^{-1})*	Specific gravity (g cm^{-3})	Dry matter (%)	Tuber number (hill^{-1})	Tuber mass (g hill^{-1})*	Specific gravity (g cm^{-3})	Dry matter (%)
Stage								
Early bud	16.2a	681(84)a	1.055a	15.3a	17.01a	752(77)a	1.052a	14.6a
Full bud	17.6a	671(82)a	1.058a	16.0a	17.41a	655(82)b	1.058a	15.9a
SEM	0.88	30.1	0.002	0.46	0.57	31.1	0.002	0.45
Rate (g a.i. ha^{-1})								
0 (control)	16.1a	681(80)a	1.053a	14.9a	16.1a	610(79)b	1.047b	13.6b
250	19.0a	750(84)a	1.058a	15.9a	18.0a	693(82)ab	1.054ab	15.1ab
500	15.0a	595(85)a	1.057a	15.7a	17.3a	723(82)a	1.058a	15.9a
750	17.2a	678(84)a	1.059a	16.2a	17.5a	790(84)a	1.061a	16.5a
SEM	1.25a	42.6	0.003	0.65	0.81	37.0	0.003	0.64

* Figure in parenthesis represents the percentage of tubers larger than 50 g.

SEM: Stand error of the mean.

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

Table 10.6 shows the effect of MCPA and PBZ on dry matter production and allocation to the different organs of potato grown under field conditions. Application of MCPA at early and full flower bud stages did not affect the dry mass of the different organs. Regardless of the concentration, MCPA reduced total biomass yield by about 11% compared to the control treatment. MCPA did not affect leaf, root or tuber dry mass, but decreased stem dry mass by about 27% l.

Table 10.6. Total biomass production (per hill) and allocation to the different plant components after a single application of MCPA or PBZ under field condition

Treatment	Leaf (g)	Stem (g)	Fruit (g)*	Root & stolon (g)	Tuber (g)	Total biomass (g)
MCPA						
Early bud	39.3(26)a	18.8(12)a	10.3(7)a	6.3(4)a	77.1(51)a	151.8a
Full bud	35.3(24)a	17.9(12)a	10.2(7)a	5.4.(4)b	77.0(53)a	145.8a
SEM	1.32	0.66	0.17	0.27	1.91	2.60
0 (control)	39.8(26)a	23.0(15)a	10.3(7)a	6.1(4)a	74.8(49)a	154.1a
250 (g ha^{-1})	36.1(26)a	17.2(12)b	00.0(0)b	5.6(4)a	79.5(57)a	138.4b
500 (g ha^{-1})	36.3(26)a	15.4(11)b	00.0(0)b	5.6(4)a	81.9(59)a	139.2b
750 (g ha^{-1})	37.0(28)a	17.8(13)b	00.0(0)b	6.2(4)a	71.9(54)a	133.0b
SEM	1.87	0.94	0.23	0.39	2.70	3.67
PBZ						
Early bud	29.9(21)a	15.4(11)a	11.8(8)a	6.7(5)a	80.0(55)a	143.8a
Full bud	34.1(23)a	19.5(12)a	11.7(8)a	6.0(4)a	81.2(54)a	152.5a
SEM	1.73	1.07	0.23	0.32	1.49	2.69
0 (control)	37.0(24)a	21.1(14)a	11.9(8)a	7.6(5)a	75.1(49)b	152.7a
250 (g ha^{-1})	29.9(22)b	15.1(11)b	00.0(0)b	6.0(5)b	82.2(62)a	133.3b
500 (g ha^{-1})	30.3(22)b	17.9(13)ab	00.0(0)b	6.3(5)ab	80.2(59)ab	134.8b
750 (g ha^{-1})	30.9(23)ab	15.7(11)b	00.0(0)b	5.7(4)a	84.6(62)a	136.9b
SEM	1.73	1.52	0.32	0.45	2.11	3.80

Figures in parenthesis represent percentage of the total biomass.

* Mean values for the rates of MCPA and PBZ are the average of the three replications.

SEM: Stand error of the mean.

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

The stage of application did not affect the impact of PBZ on dry matter production and allocation amongst organs. Irrespective of the concentration, PBZ treatment reduced total biomass yield by about 12%. Of the total carbon fixed, PBZ treated plants allotted 22% to the leaves, 12% to the stems, 5% to the roots and 61% to the tubers, while the untreated ones partitioned 24%, 14%, 8%, 5% and 49% to the leaves, stems, berries, roots and tubers, respectively.

10.5 DISCUSSION

The relatively poor fruit set observed in the control treatments could be due to high temperatures during the growing period. High temperatures during flowering may inhibit pollen tube growth and fertilization and cause abscission of flowers (Howard, 1970). In both experiments, MCPA controlled berry set by promoting flower bud abscission before flower unfolding. This could be attributed to inhibition of cell division and elongation. MCPA belongs to the growth regulator herbicides that have multiple sites of action in the plant and disrupt the hormonal balance as well as protein synthesis, thereby causing growth abnormalities (Ashton & Crafts, 1981). Veerman & Van Loon (1993) reported that application of MCPA (500 or 750 g ha^{-1}) at early or full flower bud stage reduced berry number and seed number per berry. Application of MCPA at early bud stage is ideal for decreasing berry set (Wedgwood, 1988; Veerman & Van Loon, 1993). Albeit not statistically significant, early application of MCPA consistently increased tuber yield in the greenhouse and field trials at the University of Pretoria.

Reproductive development includes the processes from flower bud initiation to fruit development (Pharis & King, 1985). There is evidence indicating that GA is involved at various stages of reproductive development, and that GA application can influence different stages of the process (Mamat & Wahab, 1992; Robers *et al.*, 1999, Brooking & Cohen, 2002). PBZ treatment promoted flower bud abscission and prevented berry set in both trials. This may be linked to a reduction in endogenous GA levels. Gibberellins are involved during early flower bud development prior to anthesis (Pharis & King, 1985). In flowers of dicotyledonous species a transitory increase in GA content prior to anthesis has been observed, suggesting that GA is involved in either or both flower opening and anthesis (Pharis & King, 1985; Sagee & Erner, 1991). Inhibitors of GA biosynthesis such as CCC and AMO-1618 block flowering in a number

of long day plants, some short day plants, and some cold requiring plants. This effect can be reversed by GA treatment under both short and long day conditions (Zeevaart, 1964; Vince-Prue, 1985).

Since the plants were grown under relatively high temperatures that encourage excessive top growth the untreated plants exhibited higher haulm dry mass than the treated plants. High temperatures decrease the partitioning of assimilates to the tubers and increase partitioning to other parts of the plant (Wolf *et al.* 1990; Vandam *et al.* 1996). MCPA and PBZ treatments influenced total biomass production and assimilate partitioning. Tubers were the dominant sinks that attracted the highest proportion of the dry matter. This dominance may be linked to lower endogenous GA levels in tuber tissue in response to the treatments. Menzel (1980) and Mares *et al.* (1981) reported that exogenous application of GA_3 inhibited tuber formation, decreased tuber sink strength and encouraged shoot and stolon growth. The increase in tuber yield as well as dry matter content in response to the treatments may partly be due to the absence of competition between developing tubers and berries. Manually removing flowers and berries increased tuber specific gravity and dry matter content (Tsegaw & Zelleke, 2002). The observed strong positive correlation between specific gravity and dry matter content indicates that specific gravity is a true indicator of dry matter content of tubers.

10.6 CONCLUSION

The greenhouse and field trials demonstrated that MCPA and PBZ effectively prevented flower formation and berry set in potato without negatively affecting tuber yield and quality. Application of the two chemicals during the early flower bud stage gave slightly higher tuber yields than late application. The trials were conducted using only one cultivar and under sub-

optimal conditions for berry formation, therefore, further field trials must be conducted to formulate legitimate recommendations. Detailed field trials using cultivars with different degrees of flowering and berry formation will be conducted under tropical highland conditions in Ethiopia.

CHAPTER 11

GENERAL DISCUSSION

Problem areas identified and initiation of the experiments

Nature has designed a few food crops that are capable of nourishing mankind, and of these the potato is one (Talburt & Smith, 1967). Potato produces good quality protein and more calories per unit area per unit of time than any other major food crop (Swaminathan & Sawyer, 1983). In Ethiopia the great potential of the crop has not been adequately exploited as is clearly illustrated by the low national yield (10 ton ha^{-1}) and small area cropped to potatoes (36, 736 ha). A number of problems are responsible for the situation, of which two are addressed in this thesis.

1. Farmers in the eastern part of Ethiopia exports a variety of vegetable crops to Djibouti and Somalia of which potato is number one in volume of export and income earning. The highlands of the region are densely populated and the average land holding is estimated at about 0.25 ha per farmer. Since the majority of the population is greatly dependent on cereals as major source of food, most of the land is used for cereal production. Despite the potential of potato as a cash crop, production has been restricted due to shortage of land. Although there are huge virgin land resources in the lowlands of the same region, potato cultivation has not been practiced due to the fact that the prevailing high temperature inhibits tuberization. There is a clear need to develop appropriate technologies to introduce potato culture to the lowlands.

2. A lack of potato cultivars adapted to the different agro-ecological zones of the country is one of the problems accounting for low yields. To tackle this problem, the Potato Improvement Program of Alemaya University has been established with the major objective of developing adaptable and high yielding potato cultivars with good resistance to the biotic and abiotic stresses of the eastern part of the country. To achieve this the program has been introducing germplasm from the International Potato Centre (CIP) and testing it across locations. Most of the promising genotypes bloom profusely and some of them set berries under the growing conditions of the highlands of Eastern Ethiopia. The effect of flowering and berry set on growth, tuber yield and quality of potato is the second topic addressed in this thesis.

The need for plant growth manipulation

Lowland tropical regions are characterized by high temperatures that limit successful potato cultivation (Midmore, 1984). Unfavourably high temperatures promote foliage growth, decrease net photosynthesis, reduce assimilate partitioning to the tubers, and increase dark respiration (Gawronska *et al.*, 1992; Hammes & De Jager, 1990; Levy, 1992; Menzel, 1980; Thornton *et al*., 1996). There is evidence that the inhibitory effects of high temperatures are mediated through the production of high levels of GA-like compounds known to inhibit tuber formation (Menzel, 1983). Previous studies showed that the hormonal balance controlling potato tuberization could be altered using GA biosynthesis inhibitors (Bodlaender & Algra, 1966; Menzel, 1980; Simko, 1994). PBZ is a triazole plant growth regulator known to inhibit GA biosynthesis and prevent abscisic acid (ABA) catabolism through its interference with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidation pathway (Rademacher, 1997). It was

hypothesized that by reducing GA biosynthesis, PBZ can improve potato productivity in the lowland tropics.

Response of potato to PBZ

The response of potato to foliar and soil applied PBZ was tested under non-inductive greenhouse conditions and in field trials in eastern Ethiopia as reported in Chapters 3, 4, 5, 6 and 7. PBZ increased chlorophyll *a* and *b* content and net rate of photosynthesis while reducing shoot growth, plant height, stomatal conductance, the rate of leaf transpiration, and tuber number per plant. It enhanced early tuber formation and delayed the onset of leaf senescence, and decreased the partitioning of assimilates to the leaves, stems, roots and stolons while increasing partitioning to the tubers. Growth analyses demonstrated that PBZ decreased leaf area index and crop growth rate while increasing specific leaf weight, tuber growth rate, net assimilation rate, and partitioning coefficient (harvesting index). PBZ treatment extended the dormancy period of the tubers. Although PBZ decreased crop growth rate, it increased tuber yield by about 58% and dry matter content of tubers by 7% over the control, probably due to the interplay of early tuber initiation, higher tuber growth rate, enhanced net photosynthesis and the diversion of more dry matter to the tubers. Balamani & Poovaiah (1985) and Simko (1994) also reported an increased tuber dry mass per plant in response to PBZ. PBZ increased tuber N, Ca and Fe concentrations while reducing P, K and Mg contents. For most of the parameters considered no significant differences were observed between methods of PBZ application. The investigation showed that PBZ is effective in suppressing excessive top growth and favouring assimilate partitioning to the tubers, thus improving tuber yield and quality. The PBZ-induced yield and quality improvement obtained in the lowlands of eastern Ethiopia may be an important step towards designing viable potato production programs for this region.

PBZ induced anatomical and morphological changes in potato (Chapter 4), which have not been clearly documented previously. The green colour of the leaves intensified because of increased chlorophyll *a* and *b* content in response to PBZ treatment. Increased leaf thickness is attributed to a thicker epicuticular wax layer, and elongated and thicker epidermal, palisade and spongy mesophyll cells. PBZ also increased leaf thickness in *Chrysanthemum* (Burrows *et al.,* 1992), maize (Sopher *et al.*, 1999), soybean (Hawkins *et al.,* 1985) and sugar beet (Dalziel & Lawrence, 1984). The increase in stem diameter was due to the formation of thicker cortex, well-developed vascular bundles, and a larger pith diameter. An increase in the thickness of the cortex and the induction of more secondary xylem vessels in response to PBZ treatment increased the root diameter. PBZ remarkably increased starch synthesis as clearly demonstrated by the deposition of starch granules in stem pith cells and cortical cells of the stem and root. PBZ treatment also increased root starch content in maize plants (Baluska *et al.,* 1993) and in the leaves, stems, crowns and roots of rice (Yim *et al.*, 1997). An understanding of the effect of PBZ on anatomical features and physiological processes can contribute greatly to our understanding of plant growth processes, and to the utilization of PBZ and similar compounds to manipulate growth.

Growth and productivity of potato as influenced by cultivar and reproductive growth

To investigate the effect of cultivar, and flower and fruit development on the growth, tuber yield and tuber quality, non-flowering, flowering and fruiting plants of four cultivars were evaluated in the sub-humid tropical highland of eastern Ethiopia (Chapter 8 and Chapter 9). Cultivars exhibited differences with respect to leaf stomatal conductance, rate of transpiration, photosynthetic efficiency, tuber yield, dry matter content, and nutrient composition. This

variability may be useful for the selection of cultivars characterized by high rates of net photosynthesis, suitable for processing or table consumption, and cultivars with reduced rates of transpiration more adaptable to moisture limited areas.

The presence of berries increased leaf stomatal conductance and rate of leaf transpiration, which may limit the productivity of the crop under moisture deficit conditions. Fruit development reduced leaf area index, tuber growth rate, assimilate partitioning to the leaves, stems, and tubers, and promoted early plant maturity. Although berry development increased photosynthetic efficiency, net assimilation rate and crop growth rate, it decreased final tuber yield and dry matter content due to the diversion of assimilates to the developing berries. Flowering and berry set restricted vegetative growth and decreased the partitioning of assimilates to the tubers thereby reducing tuber yield and dry matter content. Bartholdi (1940) reported that sexual reproductive growth reduces vegetative and tuber growth. In an investigation on the effect of flowering and berry set in *Solanum demissum Lind*. ProundFoot (1965) observed that reproductive growth significantly reduced tuber yield. Flower removal increased tuber yield and increased dry matter contents (Jansky & Thompson, 1990, Tsegaw & Zelleke, 2002).

The need for chemicals to prevent flowering and berry set

The current study as well as a previous investigation conducted at the same experimental site using the same cultivars (Tsegaw & Zelleke, 2002) demonstrated that berry set restricted vegetative growth and decreased tuber yield and dry matter content. Hence, simple and economical means to control flowering and berry set in potato should be investigated. Accordingly, greenhouse and field experiments were conducted with a major objective of studying the effect of MCPA and PBZ on flowering, berry set and biomass production, yield

and quality of potato (Chapter 10). Both MCPA and PBZ completely prevented flowering and berry set without negatively affecting yield and quality. PBZ treatment at early flower bud stage resulted in a higher tuber yield than application during the late flower bud stage. Wedgwood (1988) achieved best control of berry production in potato with a combination of MCPB and Bentazone applied at the full foliage to flowering stage. Application of MCPA at early flower bud stage reduced berry set, according to Veerman & Van Loon (1993). The study demonstrated that a single foliar spray of MCPA or PBZ at the early flower bud stage at a rate of 250g a.i. ha^{-1} was effective to inhibit flower formation and prevent berry set.

Aspects that need further investigation

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

1. There are restrictions on the utilization of products from PBZ treated plants. For instance, a residue of 0.5 mg PBZ per kg fruit is the internationally acceptable standard for mango fruit (Srivastava & Ram, 1999). The residual levels of PBZ in potato should be established before the use of the chemical for commercial purposes can be considered.
2. It has been observed that PBZ application increased the dormancy of the tubers. The effect of PBZ on dormancy characteristics and performance of seed tubers from treated plants must be investigated.
3. PBZ enhanced the photosynthetic rate as well as starch deposition. The mechanisms how PBZ affects these processes are not well understood and it needs further study.
4. The response of potato to prohexadione-calsium, a new plant growth retardant with low toxicity and limited persistence (Owens and Stover, 1999), and similar new growth

regulators, ought to be investigated in order to ascertain whether more effective or potentially safer products than PBZ are available.

5. Flowering and berry set reduced the productivity of potato by reducing both tuber yield and dry matter content. The negative effect of reproductive growth on the productivity of potato must be considered in cultivar selection strategies. Chemical means of preventing flowering and berry set need to be evaluated at farm level.

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