COMPARISON OF PRODUCTION SYSTEMS FOR POTATO (*Solanum tuberosum* L.) MINITUBER PRODUCTION WITH DIFFERENT CULTIVARS

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ABSTRACT

The purpose of these studies is to determine the effect of different production systems (Aeroponic (AP), Deep flow (DF), Nutrition film technique (NFT) and Bark) on production of first generation (prebasic) potato (*Solanum tuberosum* L.) minitubers with different cultivars (Russet Burbank, Rua, Kenebec, Atlantic and Diésrée). Prebasic tubers usually multiplied in a greenhouse in a bark medium. Replacing the bark based system by a highly intensive aeroponic system is viable. The aeroponic system significantly produced higher tuber numbers per plant and produced a higher percentage (82%) of 1 –2g size tuber than other systems evaluated.

In aeroponic and deep flow systems, the time to tuberization is higher. When tuberization is delayed, stolons grow continuously in the deep flow and aeroponic systems. In hydroponic systems a developing stolons did not encounter sufficient mechanical resistance to limit growth, they grew extremely vigorously and very wide of diameter (1.92- 2.42 mm). When roots and stolons filled the aeroponic and deep flow containers some root rustication occurred then tubers were initiated.

Acid treatment efficiently synchronised tuberization in the aeroponic systems, tubers were visible four days after acid treatment. Optimum acid treatment depends on the specific cultivar or stage of stolon development. Rua produced the highest yield per plant and highest tuber number per plant. The pH and treatment time significantly affected the tuber number per plant. Acid treatment at pH 3.5 produced the highest tuber number (19.8) per plant.

Minitubers show a dormant period immediately after they are harvested. Application of GA is a useful method to break minituber dormancy. Fifty percentage of the GA treated tubers sprouted within 20 days after treatment, while untreated control took 37 days. Overall, sprouting was highest for 100 ppm treatment, but was not significantly different

from 30 ppm. The highest and lowest sprouting cultivars were Russet Burbank and Atlantic respectively.

Potato cuttings can be propagated in an aeroponic system. Application of IBA treatment at 0.1 to 0.3% significantly induced root formation and root growth. About 80% of the IBA treated cuttings rooted and produced longer roots than untreated cuttings.

From these studies we found the aeroponic system to be suitable for minituber production as well as the propagation of potato cuttings. Production of high tuber number per plant in aeroponic systems will reduced the production cost and reduce the number of field multiplications required before final cropping.

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Chapter One

General Introduction

Potato is one of the world's most important food crops, being placed next to wheat, corn and rice in total production. Potato is a reliable crop which can tolerate a wide range in environmental conditions, with various production methods. The maintenance of a potato production system is heavily dependent on a adequate and continuous supply of high quality seed tubers. Due to its ability to produce a high volume of tubers per unit area, manipulation of production of planting materials other than conventional seed tubers may contribute greatly to the world food supply.

The nutritive value of potato, in terms of protein and carbohydrate per hectares, is better than that of any other major crop (including maize, beans, peas, wheat) except soybeans. Although production in most developed countries has remained almost static, has or fallen slightly in recent years, in developing countries potato production has risen rapidly. In New Zealand, as an example, the area planted in potatoes has fallen from 15,635 ha in 1899, to the present total of about 10,122ha, (FAO 1990-1998) but yield per hectare has increased continuously since 1899 (Genet, 1985). In 1989, 9213 hectares were planted and produced 290,000 tonnes of tubers.

Table- 1-1 Potato production In New Zealand past 30 years

Year	1966	1976	1986	1996
Area (ha)	13000	9053	8800	10122
Yield (t / ha)	21.10	26.15	31.13	45.05

FAO 1990-1998

Production total in New Zealand Asia, Europe and the world potato production from 1995 to 1997 are listed in Tables 1-2, 1-3, 1-4 and 1-5.

Potato can be attacked by many viral, bacterial and fungal diseases- and the initial seed stock, therefore, needs to be protected from these pathogens. Vegetatively propagated cultivated potato always seems to be a source of tuber- borne diseases, which lead to quick spreading of a disease in the seed stock. Therefore, it is important to monitor the routine production of disease-free seed tuber in order to maintain adequate yield. To control this problem, researchers introduced alternative planting material for potato production. For example, stem cutting or apical meristem culture (micro-propagation) can reduce the potential tuber diseases problem as well as increase the seed potato production (Goodwin *et al.*,1980; Hussey and Stacey,1981).

Table 1-2. Potato production in New Zealand

Regions	Year		
New Zealand	1995	1996	1997
Area (HA)	10,588	10,122	10,100
Yield (MT/HA)	47.7	45.6	45.0
Production (MT)	476,800	455,700	450,000
Seed (MT)	47,680	45,570	45,001

Table 1-3. Potato production in Asia

Regions	Year		
Asia	1995	1996	1997
Area (Million HA)	5.7	6.04	5.85
Yield (MT/HA)	14.8	14.9	15.1
Production (Million MT)	84.62	89.95	88.21
Seed (Million MT)	5.99	6.01	6.11

Table 1-4. Potato production in Europe

Regions	Year		
Europe	1995	1996	1997
Area (Million HA)	3.85	3.67	3.55
Yield (MT/HA)	24.39	21.09	24.59
Production (Million MT)	81.33	90.49	86.78
Seed (Million MT)	8.17	8.34	8.34

Table 1-5. Potato production in World wide

Regions	Year		
World	1995	1996	1997
Area (Million HA)	18.43	18.45	18.22
Yield (MT/HA)	15.5	16.7	16.6
Production (Million MT)	285.27	306.00	302.50
Seed (Million MT)	35.61	35.74	35.86

FAO 1990-1998

In terms of seed potato production, micro-propagation can be further manipulated as an alternative end-product of micro-propagation as microtubers. This can be done by allowing *in vitro* plantlets to grow under inducing conditions to produce microtubers which are particularly convenient for handling, storage and transport of germ-plasm. Furthermore, unlike *in vitro* propagated plants, they do not need a hardening period in a greenhouse and may be adapted to some form of large-scale mechanized planting.

Microplants can also be planted out in a greenhouse to obtain tubers (minitubers) which are smaller than normal seed tubers, but larger than microtubers. Additional use of microtuber and minitubers will depend on their yielding ability under field conditions (Ranalli *et al.*, 1994). Mini and microtubers, however, will be used on a large scale only if they reliably produce acceptable yields. Struik and Lommen (1990) reported that minitubers appear to be more suitable for direct field planting than micro-tubers. Minitubers are relatively easy to manage, and serve as an initial source of pre-basic seed stock for the certified seed potato

industry (Melching *et al.*, 1993). However, early studies by Christie *et al.* (personal communication) indicate that multiplication of potato by stem cuttings is a low-cost and low-risk method compared with *in vitro* methods.

The current seed potato production programme is based on a rapid multiplication scheme which combines laboratory, greenhouse, and field techniques. This involves the production of plantlets or tuberlets *in vitro* as a starting-point in its seed potato production programme followed by a second multiplication in high-density beds in greenhouse, and by several generations in field multiplication, before the seed is used for commercial potato production. When transplanting the *in vitro* plantlets to the greenhouse or field, there is some limitation in the survival of plantlets. Water stress, high light intensity and fungal infections depress survival when plants are transferred from an *in vitro* environment to a greenhouse or field.

The yield of plantlets is low because each plantlet produces only 3-4 mini-tubers and, therefore, it is essential to multiply nuclear tubers for several generations in open fields to increase the seed production. This procedure may increase the possibility of disease infection and also can delay the seed multiplication if the environmental conditions permit only a single crop season per year, as in New Zealand. These problems can be overcome by producing large numbers of mini-tubers in a basic seed production programme (Vodenik and Jenko, 1992).

Micro-propagation can be used to produce many plants quickly, and reduce the number of field multiplication, but this system is costly and risky. Ranalli *et al.*, (1990) reported that microplants produce more stolons and initiated tubers over a longer period. Under a greenhouse-based mini-tuber production system, single harvesting will give greater size and develop variations in the tubers. Multiple harvesting is one method both to increase the number of tubers per plant, and to produced even- sized tubers. Lommen and Struuik (1992) have reported that minitubers were produced in large quantities by repeated harvesting of tubers from *in vitro* propagated plantlets and transplanting in greenhouse at high plant densities. In a basic seed production programme, the number of field

multiplication can be reduced by producing a large number of minitubers (Vodenik and Jenko, 1992). This minimizes the risk of virus contamination of seed tubers.
The goal of this study is to examine minituber production in different systems such as Bark, Deep flow, NFT and Aeroponics to determine the best method of increasing production.

1. 2. Objectives

1) Develop a propagation system for the rooting of potato cuttings in aeroponics systems.
2) Evaluate the use of different production systems for minituber production.
3) Develop a system to synchronize the tuber initiation.
4) Develop a dormancy-breaking strategy.

Chapter Two

Literature review

2. 1. Potato production

2.1.1. Production of potato planting materials

Different kinds of planting materials are used for potato production throughout the world. The type of planting materials depends on the quality and availability of the planting materials. High-health planting materials are very important for high yield.

2.1.1.1. Seed potato

Since 1588, people have used potato tubers for both propagation and production. Using potato tubers for propagation allows disease build-up and incurs high cost due to continuous usage of tubers, storage and transport of tubers respectively. Almekinders *et al.*, (1996) stated that the cost of seed potato is very high because multiplication rates are low, storage and transport costs are high and vegetative propagation is constrained by the carry-over of pathogens. In warmer climates the constraints of seed tuber based systems are more serious, and the costs of healthy seed tubers can account for from 50 to 70% of the total production costs (Horton & Sawyer, 1985).

2.1.1.2. True potato seeds

The production of potato tubers using the True Potato Seed (TPS) technique is a relatively new technology of economic advantage to developing countries (Yilma, 1991). True potato seed technology is increasing in popularity in many developing countries where potato production is limited by the poor quality of seed tubers and high cost of healthy seed tubers (Almekinders *et al.*, 1996). These factors are barriers to the introduction of potato into new areas where it could be an efficient food crop. The use of TPS in a potato production

system can alleviate these problems. Planting one hectare takes only 200gms of true potato seeds as compared to 2 tonnes of tubers. The theoretical advantages of the TPS technology use of seed tuber production are lower cost of planting material, better quality seed tubers, easier storage and transport (Almekinders *et al.,* (1996). However in developed countries, well-performing seed tuber programmes, productivity and legislative restrictions are difficult to beat by TPS (Almekinders *et al.*, 1996). TPS can be sown directly in the field or in nursery beds, and seedlings grown from TPS can be left in place or transplanted (Wierssema, 1986 and Malagamba 1988). TPS technology is site specific and not readily transferable between countries requiring site specific research and development (van der Zagg, 1987), because the seedling survivals depend mainly on environmental condition of the site. van der Zagg (1990) states that TPS technology can compete successfully with conventionally- multiplied colonal material, and may compete successfully with the more expensive micro and mini-tubers.

2.1.1.3. Cuttings

Propagation of potato stock by stem cuttings was developed in the 1960s as a means of eliminating bacterial and fungal pathogens normally carried over by tuber propagation (Roy *et al.*, 1995). To expand potato production in developing countries cuttings are used as a source of good quality planting material, but simple, and low cost methods are required for rooting and establishment. Cutting survival and root length are not affected by the size or age of the cuttings, but treatment with rooting hormones increases the number of roots initiated above the cut end of the stem (van der Zaag & Escobar, 1990). 2 to 3 cm long apical cuttings with simple leaves have rooted successfully (Goodwin *et al.,* 1980). Light and temperature are important factors which affect root initiation and growth of the cuttings (Demagante & van der Zaag, 1988). Whereas 95% of ground cover occurred within four weeks after transplanting cuttings, *in vitro* plantlets can take 6 to 7 weeks to reach 95% ground cover (Roy *et al.,* 1995).

Sprout cuttings from seed tubers can be used as planting material for the *in vitro* technique (Hussey *et al.*, 1981) and greenhouse propagation. Van Ho *et al.,* (1988) found that

segments of sprout cuttings also developed roots and shoots equivalent to whole sprouts-and this method can be used to produce more potato seedling from a tuber

2.1.2. Rapid multiplication techniques for seed potato production

Rapid multiplication techniques are used in most of the potato-producing countries to produce healthy seed potato. However, it takes quite a number of growing seasons to generate sufficient planting material for table potato production starting from one seed tuber. In the traditional method of multiplication, seven or more generations are required to produce certified seed for table potato production (Personal communication M.A. Nichols). In a basic seed production programme, the number of field multiplication can be reduced by producing a large number of minitubers (Vodenik and Jenko, 1992).

Tissue culture techniques are now routinely used in both developed and developing countries to obtain pathogen-free planting materials for potato production. Making use of the enormous regenerating capacity, and the inherent tatipolency of cells and isolated protoplasts, *in vitro* technology has been applied to produce plants on a large scale. Three *in vitro* approaches can be applied to potato propagation.

1) Culture of nodes
2) Microtuber formation
3) Somaclones and gametoclones through callus, protoplast and pollen culture

Tissue cultured *in vitro* plantlets can be used for mass production of minitubers in the greenhouse (prebasic tuber) (Lommen & Struik, 1992). The prebasic materials need to be multiplied for a few generation in open fields to increase the quantity of seed available for certification (Ranalli *et al.*, 1990).

The current rapid multiplication of seed potato programme involved following steps.

1) Laboratory-based *in vitro* propagation
2) Greenhouse-based pre-basic tuber production
3) Field multiplication of pre-basic tubers.

2.1.2.1. Laboratory- based *in vitro* propagation

Tissue culture or multiplication of potato plants *in vitro* takes place in a laboratory under completely sterile conditions. The development and application of *in vitro* plant culture technology to potato propagation enables rapid multiplication of disease pathogen-free potato plantlets (Dodds, 1988). Tissue culture techniques are more sophisticated and needs trained personnel. The end product may be either small rooted shoots or micro-tubers, large numbers of shoots can be derived from one medium sized tuber within 6 weeks (Hussey and Stacey, 1981).

2.1.2.2. Greenhouse based pre-basic tuber production

This condition requires insect proofing in order to avoid virus infection. Most seed potato programmes involve transplanting micro-propagated plantlets to pots or beds in a greenhouse. Water stress, high light intensity and fungal infections can depress survival when plants are transferred from an *in vitro* environment to a greenhouse (Ranalli *et al.* 1990). After about 15 weeks, the crop yielded 3-4 mini-tubers per plantlet depending on variety.

2.1.2.3. Field multiplication of pre-basic tubers

The pre-basic materials need to be multiplied for a few generations in open fields to increase the quantity of seed available for certification, or table potato production. Crops from minitubers intercept less light during the growing period than do those from conventional seed tubers, and produce lower tuber yields (Lommen & Struik, 1994 and Marshall & Taylor, 1990).

2.1.3. Microtuber and Minituber production

Potato minitubers and micro-tubers may be used in seed production programmes to reduce the number of field multiplications. *In vitro* propagation of potato by serial auxiliary buds

in separated nodes has long been used for the rapid multiplication of diseases-free clones and to obtain healthy tubers to used as seed (Goodwin *et al.*, 1988; Hussey and Stacey, 1981). An alternative end product of micropropagation of the potato is the small (micro) tuber produced by allowing an *in vitro* plantlet to grow under tuber- inducing conditions. *In vitro* plantlets or micro-tubers planted in a greenhouse produce a tuber called a "minituber". Mini-tubers may weigh from 125 mg upward. The average weight of minitubers however depends on the technique used for their production (Lommen and Struik, 1992). Microtubers and minitubers are particularly convenient for handling, storage and transport. Also, unlike *in vitro* propagated plants, they do not need a hardening-off period in a greenhouse.

The production of mini-tubers involves two steps

1. Microplantlet production
2. Minituber production

2.1.3.1. Microplantlet and Minituber production

Microplantlets can be produced from nodal segments, or meristem tip culture. An initial explant is derived from the sprout of virus-free tubers. The resulting plantlets are multiplied by cutting them into nodal segments which are transferred to a fresh medium every four weeks until sufficient plants for mass tuberization have grown (Ranalli *et al.*, 1988). The multiplication rate ranged from 3 to 5 per month, so that 3 to 5 cuttings could be obtained from one plantlet after four weeks' growth (Ranalli *et al.*, 1994).
Potato minitubers can be used as a propagation material for the production of high quality seed tubers in a seed programme comprising only a few field multiplications. Minitubers are usually produced by direct transplanting of whole *in vitro* plants at high density in the greenhouse (Lommen & Struik, 1992). Minitubers are relatively easy to manage, and serve as an initial source of pre-basic seed stock for the certified seed potato industries (Vodenik & Jenko, 1992). In a basic seed potato production programme, the numbers of field multiplications can be reduced by producing a large number of minitubers (Vodenik & Jenko, 1992). Yield parameters of mini-tubers can be manipulated by crop husbandry.

Minituber yield was increased by supplying nutrients or by using a square plant arrangement (Lommen & Struik, 1992). Minituber production was carried out in a peat-based medium containing the mycorrhizal fungus *Glomus intraradix.* It increased yields of most valuable size of prenuclear mini-tuber by 84%, and increased total prenuclear minituber yield by 49% when compared with conventional peat vermiculite media, plants grown in this medium had more uniform stolon development than those grown in conventional media (Niemira *et al.*, 1995).

Foliage senesced after 12 weeks and the minitubers were then harvested. Minitubers were produced in large quantities by repeated harvesting of tubers from *in vitro* propagated plantlets at 4, 7 and 10 weeks after transplanting to the greenhouse at high density (Lommen & Struik, 1992). Each plant usually produced tubers with a diameter ranging from 12 to 20 mm, and weighing from 0.4 to 4.4g. The higher frequency distribution of minituber weight was 1.5 to 2.4g and 2.5 to 3.4g (Ranalli *et al.*, 1994). Therefore minitubers appear more suitable for direct field planting than microtubers, because they are of greater weight than microtubers (Struiuk & Lommen, 1990).

2.1.3.2. Microtuber production and development

Microtubers are produced *in vitro* under sterile conditions. Microtubers begin to appear 3 weeks after transferring plantlets into the tuberization medium; some were epigeal and others hypogeal. Usually epigeal microtubers grew at the end of the shoots or were axillary. Microtubers developing within the culture medium either grew from stolons or were produced shoots, which had grown downwards into the medium. One, or occasionally two, tubers were formed on each plantlet. Hulscher *et al.*, (1996) have used jar fermentors for mass propagation of potato microtubers. They found tuberization was inhibited when the shoots are continuously submerged in the liquid medium. Medium aeration also had a considerable effect on the number of microtubers in jar fermentors.

2.1.4. Field performance

The field performance of potato plants depends on the source of mother plants and crop husbandry. Well-established plants will give a better final tuber yield than do weak plants.

2.1.4.1. Cutting

Potato plants from cuttings show lower field performance in all aspects than do plants from normal seed tubers. Conventional seed tubers give much higher yields than rooted sprouts which, in turn, give higher yields than do direct planted sprout cuttings. 3-node sprout cuttings rooted in a medium of subsoil, manure and ash provide vigorous plants, and yields from these cuttings were 5 – 30 tonne/ha (Ho *et al.*, 1987). Rooted cuttings produce fewer tubers per plant or per m^2, but tuber yield by weight was higher than or equal to the *in vitro* plantlets. There were relatively few tubers on rooted cuttings due to the fact that fewer nodes of the rooted cuttings were within the production medium (Roy *et al.*, 1995). Tuber and stolon formation increased significantly when more nodes were included with the cutting (Vander and Escobar, 1990).

The rooted cuttings, tuber initiation and 95% ground cover occurred within four weeks after transplanting as compared to 6 weeks and 7 weeks for the *in vitro* plantlets. This rapid growth and enhanced light interception occurred as a result of increasing photoperiod and light intensities (Ali *et al.*, 1995.; Roy *et al.*, 1995), but fewer tuber per plant or per m^2 were produced by the rooted cuttings. If there were fewer nodes of the rooted cuttings within the production medium, then relatively fewer tubers were produced (Roy *et al.*, 1995).

2.1.4.2. Microtuber and Minituber

Potato minitubers and microtubers can be used as propagation material for the production of high quality seed in a seed programme involving only a few field multiplications (Lommen and Struik, 1995). Multiplication factors can be used in evaluating the field performance of minitubers and micro-tubers. Multiplication factors can be expressed in

terms of the number or weight of the progeny tubers produced per planted tuber, or per unit planted tuber weight (Lommen and Struik, 1995). The number of progeny tubers per plant increased with the weight of the minituber planted (Lommen and Struik, 1995).

2.1.4.2.1. Plant emergence and ground cover

Plant emergence and ground cover depend on the size and physiological stage of tuber. The rate of sprouting in microtubers increased with storage time, and was greatest in the larger micro-tubers (average 0.61g) (Choi *et al.*, 1994). Field emergence increased with the size of the microtubers and was highest in microtubers stored for 30 days (Choi *et al*, 1994). Plants from microtubers emerged 8 – 9 days later than those from minitubers and 14 – 15 days later than those from normal tubers (Ranalli *et al.*, 1994). Microtubers and mini-tubers produced only one primary stem after one month of growth. (Lommen & struik, 1994.; Ranalli *et al.*, 1994). The total number of branches per primary stem was not significantly affected by the weight of the minitubers, but the proportion produced on below-ground nodes was lower in the plants from minitubers with a higher fresh weight (Lommen & Struik, 1994). The stems of micro and minitubers branched profusely to give short bushy plants (Ranalli *et al.*, 1994).

The heavier minituber gave a more regular emergence, faster ground cover after emergence, higher dry matter yields, and higher fresh tuber yields. The slower ground cover of crops from micro-tubers and lighter mini-tubers soon after emergence was probably caused by a slower haulm development per plant due to the relatively small root system and smaller amount of tuber reserve available for growth (Lommen & Struik, 1994). Also, the percentage ground cover 51 days after planting was not influenced by spacing between rows, and average values were 10.5, 37.8 and 72.4% respectively for micro, mini and normal tubers. The ground cover rate of plants from micro-tubers and mini-tubers were, respectively, 54.1% and 83.4% at wide spacing and 69.4% and 88.1% at close spacing.

2.1.4.2.2. Tuber yield

Minitubers (< 5g) from nodal cutting may not be suitable for direct field multiplication, but could be sprouted and used to propagate more minitubers in a greenhouse. It was reported (Wattimena *et al.*, 1983) that yields from *in vitro* microtubers of 0.5 – 1.0 cm diameter were equal to those produced by 40 – 60 g whole seeds; however , such microtubers were established as plantlets in the greenhouse for 2 or more weeks prior to transplanting in the field, and required a longer growing period (Ali *et al.*, 1995).

The distance between rows significantly affected the performance of micro and minitubers (Ranalli *et al.*, 1994). At close row spacing, microtuber and minitubers yielded more than at wide spacing. Tuber yield and total number of tubers per m^2 were influenced also by the tuber sources and row spacing (Melching *et al*, 1993.; Ranalli *et al* 1994., Ali *et al.*, 1995.; Lommen *et al.*, 1995). However, tuber yield per stem decreased with increasing planting density. At a closer distance between rows, micro-tubers produced more than the other two propagule sources. At wider spacing between rows, the total tuber number as well as the tuber yield increased progressively from microtubers to minitubers to normal tubers.

The number of progeny tubers per plant increased with the weight of the minitubers planted. The weight of progeny tubers produced per plant increased more clearly and consistently with the mother tuber weight (Lommen & Struik, 1995.; Thornton & Neundorfer, 1986). Encapsulation of the minituber in peat lite mix, closer seed tuber spacing and 2 mini-tubers per hill significantly increased the total yield (Melching *et al.*, 1993).

2.1.4.3. Tuber distribution

The tubers from a single potato plant vary greatly in yield and in their size distribution. This can be described by their number and their average size and its variation. These components are mutually, and closely, related (Struik *et al.*, 1991). The variation is caused partly by stolon characteristics, including their date of initiation, position and size. At any

given time the tubers on one stem vary in size. The differences in size are partly related to differences in the date of tuber set, and in the rate of growth. However, it is commonly believed that the rate of growth of each individual tuber fluctuates (Gray, 1973.; Ahmed and Sagar, 1981.; Struik *et al.*, 1988). Shortly after tuber set, there seems to be some relationship between the sink strength and its size of individual tuber (Engels and Marschner, 1986). However, the relationship weakens during the later stage of growth (Opparak, 1985), because the sink strength of a tuber no longer depends on its size alone, but also on other characteristics such as the levels of hormones, the mineral composition and the turgor potential which are all associated with the tuber growth rate (Engels and Marschner, 1986). The growth characteristics of individual tubers may also vary since they are exposed to different conditions because of differences in position or growing period (Struik *et al.*, 1991).

Struik *et al.*, (1991) noted that factors, which affect the tuber growth, could be divided into four groups.

a. The position, date of initiation and size of the stolon to which the tuber is attached.
b. The date of initiation of the tuber, and its initial activity.
c. Internal tuber characteristics, such as turgor potential, hormone concentration and enzyme activities.
d. Variation in external conditions over time or within the plant.

The tuber size distribution was influenced also by the source of the planting material. Micro-tubers produced 74% in tuber class <36mm, 25% in class 35 – 55mm, and 1.5 % in class 55 – 80mm. The plants obtained from minitubers produced 50.6%, 46.8% and 2.7%, respectively in the three classes. Plants obtained from normal tubers produced most tubers in the 36 – 55mm class (59.7%) (Ranalli *et al.*, 1994). Plants from conventional seed tubers produced more progeny tubers, and a higher tuber weight than plants from minitubers, because they had higher numbers of stems than did plants from minitubers (Lommen & Struik, 1995). These initial differences in the rate of tuber growth may be important for the final tuber size distribution. Tuber-specific factors such as the position and activity of the tuber also play a role, but the date of initiation of an individual tuber is not crucial.

2.1.5. Tuber dormancy

Potato tubers show a dormant period after they are harvested, and therefore cannot be planted immediately. The length of dormant period is cultivar specific (Leclerc *et al.*, 1995). Mini-tubers also show dormant periods for some time after harvesting (Lommen, 1993a). The dormant period (days from harvest to 50% sprouting) was longer with lower than with higher weight tubers (Leclerc *et al.*, 1995 and Lommen, 1993a). The duration of dormancy was also significantly (positively) related to the date of tuber initiation, and to the position of the tuber on the plant during its growth, tuber weight and cultivar (Lommen. 1993a). Environmental conditions during the growing period have significant effects on the post- harvest physiological ageing of tubers. For example, temperature and daylength have a pronounced effect on this process (Susnoschi, 1981 and Sorce *et al.*, 1997). Also, location and altitude of the growing site (Susnoschi, 1981) and degree of maturity of harvested tubers (Hutchinson, 1978b; van Intersum, 1992b) affect the length of dormancy. Tuber dormancy in seed potatoes varies between cultivars (van Loon, 1987, and Susnoschi, 1981). Shortage of soil moisture during the growing period (Cho *et al.*, 1983, Karafyllidis *et al.*, 1996) and pre and post-harvest treatment (Hutchinson, 1978a and van Ittersum 1992a) affect the dormancy. It is affected also by the level of indigenous growth regulators within the tuber (Coleman, 1987) and physiological age of the mother plants (Lommen, 1993a).

Application of controlled atmospheres (CA) based on high levels of carbon dioxide, oxygen and nitrogen were effective in removing dormancy. A reversible and rapid reduction in the endogenous inhibitor, abscisic acid, as well as increased sugar levels was associated with CA effects (Agriculture and Agri Food Canada: http://res.agr.ca/fred/home/texts/achieve.htm). The shortening of the dormancy of minitubers by cold storage periods after curing is consistent with the positive effect (Lommen, 1993b).

2.1.6. Different kind of production systems

Different kinds of production systems are used in the world for the intensive production of vegetable and ornamental crops. Usage of different systems depends on the knowledge and economic condition of the people. In recent years, the use of hydroponics systems has increased for several reasons. The use of soil-less substrates is gradually replacing steam-disinfected soil in the greenhouse industry (Gullino and Garibaldi, 1994).

2.1.6.1 Hydroponic Systems

Hydroponics, the method of growing plants without soil, presents a feasible alternative to conventional farming in areas which are short on water supply and limited in agricultural soil. Hydroponics is now defined as the "science of growing plants without the use of soil, but by use of an inert medium, such as gravel, sand, peat, vermiculite, pumice or sawdust, to which is added a nutrient solution containing all the essential elements needed by the plant for its normal growth and development". There are several excellent reasons for replacing soil with a sterile medium. Soil-borne pests and diseases are immediately eliminated, as also are weeds. Since many hydroponic methods employ some type of medium which contains organic material like peat or sawdust, it is often termed "soil-less culture", while water culture alone would be true hydroponics.

A regular supply of irrigation water is an important factor for the production of good quality seed potato, but irrigation is not always possible in many parts of the world. Hydroponics systems can be used to minimize the water loss and ensure a regular supply of water for potato plants. Water stress in potato during the growing period decreases yield and the number and size of tubers (Karafyllidis *et al.*, 1996). Therefore, the yield potential of seed potato can be improved by an adequate supply of water. Plants grown in soil-less culture have higher concentration of Ca, Mg and K than soil-grown plants, but similar concentration of P and Fe. There were no significant differences between aeroponics and NFT or deep flow grown plants (Chitkay and Kon, 1995).

The microbiological point of view of hydroponics systems, and knowledge about metabolic processes in the nutrient solution and the micro-organisms involved are still incomplete (Berkelmann *et al.*, 1994).

2.1.6.1.1. Deep flow culture

Deep flow systems are ideal for optimizing root absorption by regulating the temperature, oxygen and Electro conductivity (EC) of the nutrient solution by adjusting the volume and nutrient composition of the feed (Ho and Adams, 1995). Continuous replenishment of nutrients in a hydroponics environment ensures efficient nutrient uptake, which is the key to growing plants at relatively low nutrient concentration.

The most practical method for adjusting the nutrient supply in relation to demand in hydroponics systems is by measuring the total ionic concentration of the solution as the electro conductivity (EC) in the root zone. Plant development can be adjusted by modifying the EC, when the water relations of the plant are altered (Ho and Adams, 1995. Internet 1998, Source unknown). However in propagation systems, the deep flow technique has some disadvantages.

As a method for plant propagation deep flow has not become commercially acceptable because, as in the bed methods, plants can easily become infected. Also, deep-flow grown plants develop what is known as a "water root" which suffers shock when transplanted which causes a setback in normal growth.

2.1.6.1.2. Nutrition Film Technique (NFT)

Nutrient film technique was developed in the mid 1960's in England by Dr Allen Cooper, who was interested in building a low cost, large scale system to be used in parts of the world where soil quality is poor. NFT systems work by having a continuous flow of nutrient-laden solution flowing over the root system, allowing the plants to feed constantly, resulting in increased productivity and yield. NFT systems use little or no

medium, thereby keeping operating costs down, but because of the lack of medium to act as a buffer they are particularly susceptible to equipment failure. Trellising may be required when growing larger plants in NFT systems because of the lack of medium to hold the plants in place.

In the NFT system the plant roots are supported only on the base of a gully containing a shallow layer of the nutrient solution. NFT is, theoretically, sterile. However, a very rigorous hygiene schedule is necessary in order to maintain this condition throughout the cultivation (Gullino and Garibaldi, 1994).

2.1.6.1.3. Aeroponic culture

Aeroponic systems use pumps and nozzles to spray nutrient solution directly onto the root system of the plants. Aeroponic systems have shown extremely fast growth rates, and high propagation success rates (personal observation), due to the large amount of oxygen available in the systems. These systems have proven capable of propagating plants using very little growing medium in these systems reduces operating costs, but increases the risk of crop loss in the case of equipment failure. Aeroponic technology for plant propagation and growing is now a commercial reality for the greenhouse grower. Aeroponic systems can help increase yields with a great survival rate, and grow more plants in less time and space, with negligible water loss. Aeroponics should not be confused with hydroponics which is the growing of plants directly in a water and nutrient solution. Aeroponics is the process of propagation and growing plants in air. Aeroponic culture is ideal for improving the aeration of the feed and allows the modification of root temperature to optimize both root growth and the absorption process. However, depletion of oxygen is enhanced by a high root temperature, which stimulates root respiration, and by a high population of micro-organisms, which compete with the roots for oxygen.

A comparison of aeroponics (spraying with nutrient solution) and the nutrient film technique (NFT), showed that photosynthetic activity and leaf respiration were significantly higher under aeroponics than under NFT (Yang, *et al.*, 1990). Aeroponics systems resulted

in higher plant concentrations of P, K and Mg but lower concentrations of Ca than NFT, while Cu, Zn and Fe were similar in both (Yang, *et al.*, 1990). Root respiration was higher under aeroponics than under NFT, and among plants grown aeroponically it was highest (50% higher than NFT plants) in plants sprayed most frequently (1 min every 5 min). The contents of endogenous GA and zeatin riboside were higher under aeroponics, but ABA and IAA contents were lower. These differences varied between roots and leaves (Yang, *et al.*, 1990).

Maintaining a pathogen free environment in the aeroponic system is dependent on several important factors.

1. The use of pathogen-free pathogen-resistant plants or seeds
2. The highly sanitary conditions of the facility housing the aeroponic system.
3. The delivery of pure water to the system
4. The injection of sterilized micro nutrients into the system

2.1.7. Microbiological activity in soil-less culture

NFT, deep flow, and aeroponic systems are theoretically sterile. However, a very rigorous hygiene schedule is necessary in order to maintain this condition throughout the cultivation. The development from growing in border soil to soil-less cultures has not resulted in the disappearance of soil-borne diseases. Most root-infecting pathogens occur also in these new cultivation systems (Runia, 1994). Re-circulation of the nutrient solution can easily spread pathogens entering the storage tank. In a non-recirculating system, the storage tank cannot be contaminated by the nutrient solution returning from the growing area, thus the plants are less prone to rapid spread of pathogens (Zinnen, 1988). *Fusarium conidia* spread very rapidly in the circulatory system, easily contaminating the entire system. *Fusarium condia* viability in the nutrient solution, although short, is long enough to infect plants (Kegler *et al.*, 1982). *Pseudomonas solanaceraum* also was reported as a pathogen in tomato soil-less culture (Gullino and Garibaldi, 1994). High temperature and moisture seem to provide an ideal environment for *Pythium spp.* Proliferation (Favrin *et al.*, 1988). *Pseudomonas* which inhibit plant growth can easily develop in hydroponic systems (van

Peer *et al.*, 1990). In traditional cultivation some pathogens considered as minor pathogens may, in hydroculture, develop into really major pathogens. In NFT cultures of tomatoes and lettuces, *Pythium spp*, may even lead to total crop loss (Assche and Vangheel, 1994).

2.1.8. Disease management in different systems

Disease management and sanitation of nutrition solution is important in glasshouse production systems. Aeration of the nutrient solution is undoubtedly an important factor in ensuring root health, thus preventing attacks by minor pathogens (Jarvis, 1992). Generally crops can tolerate higher values of electrical conductivity (EC), thus manipulation of EC becomes a powerful tool for growth control. However, high EC values, by damaging root tips and root hairs, can encourage infection by necrotrophic pathogens (Jarvis, 1992). Ultraviolet irradiation can be applied to eliminate pathogens from the nutrition solution. Ultraviolet irradiation in the nutrient solution has shown to be effective for the control of pathogens under experimental conditions (Gullino and Garibaldi, 1994.; Runia, 1994), but destruction of iron chelate after UV irradiation, leading to iron chlorosis has been reported (Daughtrey and Schippers, 1980). Ozone, bubbled with air through a columns of nutritional solution inhibited microbial growth, under experimental conditions. In the case of ozone treatment, however, destruction of iron chelate was observed (Jarvis, 1992). Chlorinating cannot be used to sterilise hydroponics solutions, because the effective concentration of free chlorine is phytotoxic (Jarvis, 1992).

In order to prevent minor pathogens from causing such disease problems, optimising the plant's growth conditions should be carried out with utmost care. *Phytophthora, Pythium* and *Olpidium* constitute a major problem in hydroculture due to the formation of zoospores which can, either actively or passively, spread all through the cultivation system (Assche and Vangheel, 1994). Berkelmann *et al.*, (1994) have reported that genus *Pseudomonas* were detectable in large densities and seemed to play a dominant role in the case of hydroponics with recycled nutritional solutions. Taking their optimal growth temperature and their strictly aerobic metabolism into account, nutrient solutions offer optimal

conditions for an extensive propagation of *Pseudomonas* (being nitrogen-rich, oxygenous and warm).

Fallik *et al.*, (1994) have reported that hydrogen peroxide (H_2O_2) is a main compound in Sanosil-25 (48%) with 0.5% silver iron (Ag^+) as a stabilising agent Sanosil-25 is a universally applicable disinfectant which is highly effective against pathogenic bacteria, fungi, algae, viruses and amoebae. Hydrogen peroxide is effective in killing bacterial spores (Smith and Brown, 1980). <0.02% concentration of Sanosil-25 inhibits the growth of *Erwinia caratovora*, *Pseudomonas syringae* and *Xanthomonas campestris in vitro* (Fallik, unpublished results, Cited by Fallik *et al.*, (1994). To be effective, H_2O_2 must kill or inhibit the growth of any micro-organism of concern without injuring the commodity (Rij *et al.*, 1995).

2.1.9. Rooting of Potato cuttings

In developing countries, seed tubers are the major production expense in potato production. Using the potato cuttings as a source of good quality planting materials is the cheapest way of potato production in developing countries. Rooting of potato cuttings in propagation systems depends on many factors.

2.1.9.1. Role of water in rooting

In vegetative propagation from leafy cuttings, the cuttings must remain turgid until new roots form. Water stress on the cutting causes closure of the stomata. Stomatal closure affects carbohydrate gain through photosynthesis. Photosynthesis will be affected by reducing the diffusion of carbon dioxide to the chloroplast, and will indirectly effect a rise in leaf temperature. Once roots are initiated, their further growth is heavily dependent a upon good supply of photosynthate (Loach, 1988).

ABA and ethylene concentrations rise in water-stressed cuttings (Rajagopal and Anderson, 1980). ABA influences the rooting in a wide range of species. Rasmussen and Anderson

(1980) found that appearance of the roots and the basal ends of the cutting were strongly affected by the highest concentration of ABA, and IAA was activity reduced in water stress cutting. Darbyshire (1971) found that an increase in the water deficit, increased the IAA oxides activity-thus root initiation may also be decreased by low water potential. Adventitious root formation clearly involves cell growth and the synthesis of new components, and both are affected by water stress in a number of ways. Turgor pressure is essential in providing the necessary force for cell expansion. Cell expansion is important during growth of the initiated roots through the cortical tissues, but even the earliest stages of initiation involve enlargement of " competent" cells in the peri-vascular region, prior to their division (Loach, 1988).

2.1.9.2. Size and age of cutting

The cutting survival and root length were not affected by the size or age of the cuttings.(Vanderzaag *et al.,* 1990). Larger and older cuttings produced more root and slightly better shoot growth. Tuber and stolen formation increased significantly when more nodes were included with the cutting but the age of the mother plant had no effect. Nodal cutting from different parts of the *in vitro* plants had similar survival rates when planted in 2 cm deep moist promix (a peat based growing medium) at density of 500 propagules per square meter. Over 90% of the nodal cutting including the terminal cluster, rooted and were established as young plants within a 4 weeks period (Ranalli *et al.,* 1990.; Alam *et al.,* 1995).

2.1.9.3. Environmental condition for rooting

Adventitious root formation in cuttings must have 'competent' cells capable of forming root primordia and the appropriate endogenous condition to support further development. The primary environmental requirement is to minimise transpiration from the cutting which, lacking roots, cannot replace the transpired water, control the leaf temperature and promote photosynthetic production.

Light and temperature are important variables affecting the establishment of cuttings. Root initiation and growth are adversely affected by too little light-but too much light stimulates premature tuberization (Demagante & Vander Zaag, 1988). Potato cuttings can be rooted and established without the aid of hormones, by using media components which are cheap and readily available in tropical developing countries (Vander Zaag *et al.*, 1990).

2.1.9.4. Temperature:

Light and temperature are important variables affecting the establishment of cuttings. High temperature (above 26 °C) reduced the root length and shoot fresh weight compared to low temperature (average 21 °C) (Vander and Escobar, 1990). Bryan *et al.*, (1981) found that temperatures 20 to 23 °C were best for the rooting of cuttings, although Goodwin *et al.*, (1980) found that optimum temperature for rooting varied with light intensity.

2.1.9.5. The effects of leaves on rooting

The role of leaves in rooting is thought to be primarily that they provide certain nutritive materials beneficial to root formation (Ohta and Furukawa, 1975). It has long been known that the photosynthetic activity of the leaves contributes to rooting, and there has been considerable supporting experimental evidence. In *Hibiscus rosa sinensis* , it has been shown that the chief effect of the leaves in root formation is through their supply of nutritional factors to the base of the cuttings (Zimmerman and Hitchcock, 1933). Cuttings show a wide range of response reflecting the degree of induction of the intact plant. An apical cutting from a completely non-induced plant for tuberisation will develop a vigorous root system, and will undergo considerable shoot growth (Ewing, 1985). If the plant from which the apical cutting was taken received a very low level of induction over a few short days, but not enough to induce tuber formation, or over many days for only a little longer than required for tubers, then the cutting will develop fewer roots and there will be less shoot growth. Very strong induction results in a sessile tuber at the buried bud (Ewing, 1985). Sessile tubers represent the highest level of induction. Their presence is associated with greatly reduced growth in all other parts of the cutting, including both root and shoots.

Apical cuttings from highly non-induced plants will almost always fail to develop growth at the buried bud. At the other extreme cuttings from very highly induced plants almost always develop sessile tubers (Ewing, 1985).

2.1.9.6. Rooting medium

A good rooting medium must provide adequate aeration and moisture retention. Media with high N can induce rapid growth once rooting has been initiated (Vander and Escobar, 1990).

2.1.9.7. Mineral Nutrition

Mineral nutrition is one of many factors which influence adventitious rooting in cuttings. Adventitious rooting and mineral nutrition are intimately related. Root formation on stem cuttings is a multi-stage process and few studies have distinguished between between mineral effects at the various stages (Hartmann and Kester 1983). However the various stages can be reduced to two general stages consisting of 1) root initiation; and 2) root growth and development (Blazich, 1986). Mineral nutrients directly influence both root initiation, and root growth and development. The presence of minerals has other effects also which are worthy of consideration, such as leaching of mineral nutrients during mist propagation.

2.1.9.8. Influence of hormone on adventitious root formation

The number of roots initiated per cutting may be a function of the amount of auxin-like substance present in the regeneration zone (Bastin, 1966). In a number of cases, endogenous auxin content has been reported to increase in the base of cuttings during the root inducing period (Michniewicz and Kriesel 1970). IBA treatments increased the number of roots initiated above the cut end of the potato stem, but did not result in any improvement in root length (Vander and Escobar, 1990).

Trefois and Brunner (1982) found a positive correlation between endogenous auxin-like activity in Prunus spp. Cuttings and the percent of rooting of cuttings only where the auxin level was initially high at the time of auxin treatment. Endogenous auxin treatment on rooting had no efect when the endogenous auxin level was low at the time cuttings were prepared.

2.1.9.10. Genetic Effects on Adventitious Rooting

Adventitious rooting of cuttings may be directly or indirectly controlled by genetics.

2.1.10. Potato varieties

2.1.10.1. Kennebec (U.S.A. origin)

Kennebec is grown for processing into frozen chips (French fries). Kennebec tubers are round to oval, medium to large with shallow eyes and white skin and flesh. The growth period is 110 to 130 days. The plants are thick-stemmed and vigorous, and carry a dense canopy of large, dark leaves. The flowers are white. Tubers are dormant for about two months in summer or three months in winter. Kennebec tubers store moderately well but 'green' very rapidly when exposed to light This variety does not tolerate the wash-packing process during warmer months. Kennebec is prone to hollow heart, and requires less nitrogen than other varieties. The variety is suitable for crisps and French fries when grown under conditions which favour production of high specific gravity. They are less susceptible to internal heat necrosis (brown fleck) when grown in light soils under high temperatures (http://www.agric.wa.gov.au/agency/pubns/farmnote/1990/F00290.htm).

2.1.10.2. Atlantic

Atlantic has light to heavily netted, white skinned tubers. The tubers are oval to round. Production of this variety may increase as it produces good quality crisps and has a high specific gravity. This variety has resistance to potato cyst nematode, and slight scab resistance (http://www.agric.wa.gov.au/agency/pubns/farmnote/1990/F00290.htm).

2.1.10.3. Désirée

Désirée is a high yielding, red skinned, yellow fleshed Dutch variety suitable for winter planting. Its maturity is medium to late. The tubers are long, oval, smooth, with shallow eyes. Désirée was introduced as a variety, which met the market requirements in South-East Asia. The variety is prone to set breakdown (particularly after cutting) when planted in warm soils. Summer plantings have also produced a high percentage of irregular tubers with very deep eyes at the 'rose end'. Désirée needs less nitrogen fertilizer.
(http://www.agric.wa.gov.au/agency/pubns/farmnote/1990/F00290.htm)

2.1.10.4. Russet Burbank (Netted Gem)

Russet Burbank is a high yielding, high dry matter, russet skinned, white-fleshed American variety. The tubers are long, cylindrical, or slightly flattened with shallow eyes.
It is a late maturing variety which needs about 140 days to produce optimum processing yields. It has a long dormancy. For cooking qualities and uses, Russet Burbank is the preferred variety for the production of French fries by the major processors in Australia and the United States. It produces excellent light coloured French fries. In the United States it has remained an important fresh market variety since the 1900s.
(http://www.agric.wa.gov.au/agency/pubns/farmnote/1990/F00290.htm)

2.2. Potato physiology

2.2.1. Tuberization:

Potato tuberization is a physiological process which forms a storage organ. The tuber is commonly thought of as a swollen stolon tip, but any shoot apex (including apical and axillary buds, floral buds, stolon tips, and sprouts on tubers) is capable of tuberizing (Werner, 1954). Tuber induction is under photoperiod control, and the stimulus is perceived in the leaves (Gregory, 1965). The response to photoperiod interacts with many other environmental factors. Photoperiod and temperature are the main factors which

control tuberization in potato plants (Struik et al, 1989). Short day (SD) promotes tuberization, while long day (LD) or continuous light delays this phenomenon. Response to photoperiod interacts with other factors such as temperature, mother tuber physiological age, N nutrients and endogenous hormonal factors. High temperature affects tuberization only by inhibiting stolon initiation, stimulating stolon elongation or by preventing the inhibition of stolon growth.

Tuberization consists of several steps, each controlled by hormonal balances. The final step, the actual swelling of the stolon tip, is preceded by stolon formation, inhibition of its longitudinal growth, and tuber induction (Vreugdenhil and Struik, 1989). These steps are influenced by many factors.

Potato tubers initially were assumed to be roots because they are ordinarily formed underground, however botanically, they are greatly shortened and thickened stems which bear scale leaves (cataphylls), each with a bud in its axial. The usual site of tuber formation is a stolon tip. Potato stolons are diagravitropic stems, normally arising as branches from underground nodes. Tuberization takes place when elongation growth of the stolon is inhibited and when the level of the tuberization stimulus is sufficiently high(Struik *et al.,* 1989). Axillary buds of the potato plant can form stolons: diageotropic shoots with reduced leaf growth. Under favourable conditions, tubers will form on these stolons. Only under rare, specific conditions can tubers form without prior stolon initiation (Vreugdenhil and Struik, 1989).

The following developmental steps are necessary for the formation of tubers on the potato plant (Vreugdenhil and Struik, 1989).

a). Stolon induction and initiation: activation of an axillary bud on the stem base, the first visible outgrowth of this bud as a horizontally growing shoot, and the maintenance of diageotropic growth;
b) stolon growth: the elongation and branching of the stolon;
c) cessation of longitudinal growth of the stolon;

d) tuber induction and initiation: the production and translocation of a tuber-inducing stimulus, resulting in radial growth of the stolon tip. This step is associated with changes in the metabolism in the stolon tip. These steps are influenced by environmental and hormonal factors. The whole sequence of events, from stolon induction to tuber initiation, will be referred to as "tuber formation".

2.2.2. Stolon formation

Although it is influenced by both cultivar and propagule, stolon formation usually starts at the nodes closest to the mother tuber and progresses acropetally (Cutter, 1978). It is, commonly believed that long days favour stolon growth but this is only partially correct. It is of course, true that strong induction from short days and cool temperatures will reduce stolon growth through conversion of the growing points to tubers (Ewing, 1985). In cuttings, stolon induction is an intermediate stage between bud inhibition and tuberisation. Stolons can form at any lateral bud (Kumar and Wareing, 1972). They develop first at the most basal nodes and formation is favoured by darkness and high humidity (Kumar and Wareing, 1972). Tuber and stolon formation increased significantly when more nodes included with the cutting (Vander and Escobar, 1990).

2.2.3. Environmental factors affecting tuberization and stolon formation

Physiological responses to temperature, photoperiod, irradiance, nitrogen and other environmental factors, especially temperature and photoperiod will influence the tuberization of potato. Therefore, maximum tuber production requires an optimal temperature photoperiod combination. Both early and late tuber formations will shorten the growing period. Dry matter partitioning and tuber quality can be influenced by changing the microclimate around certain plant parts, and may help to improve techniques for growing potatoes in hot climates (Struik *et al.,* 1989).

2.2.3.1. Photoperiod and Spectral Quality

Under ordinary growing conditions in the temperate zones, stolons are formed on whole plants before, or shortly after, plant emergence. If stolon growth has already been initiated, exposure to short photoperiods will inhibit stolon growth because tuberization will be favoured instead. Potato plant exposure to 10-h photoperiods with day/ night temperatures of 21/ 11^{0}C increased stolon number and length compared to control plants maintained under 16-h photoperiods and 28/25 ^{0}C. It is probable that both short days and cool temperatures contributed to the increased stolon growth. Struik *et al.,* (1988) observed that, under long photoperiods, the initiation and growth of stolons was enhanced compared with short day conditions so that many potential tuber sites were created. However, a longer photoperiod delayed the onset of tuber bulking-more at higher than at lower temperatures (Vandam *et al.,* 1996). It is generally accepted that long photoperiods inhibit tuber initiation and promote potato shoot growth (Ewing and Struik, 1992). Whenever the photoperiod was increased, there was a marked decline in net carbon assimilation rate, which was most pronounced under high light condition (Sutte *et al.*, 1996.; Cao and Tibbitts, 1991). Snyder and Ewing (1989) have reported that under a long photoperiod there is an overall reduction in tuberization on cuttings. The variety "Désirée" was so sensitive to the long photoperiod that it produced no tubers with high temperatures, and almost none even with cool temperature. The longer photoperiod and high temperature caused a later onset of tuber bulking and tuber growth. Absolute tuber growth rate was not affected by photoperiod, but the effect of photoperiod on the period between the onset of tuber growth and the onset of tuber bulking is probably relevant for the number of tubers reaching harvestable size (Vandam *et al.,* 1996). Therefore, maximum tuber production requires an optimal temperature- photoperiod combination. Vandam *et al.,* (1996) concluded that a low mean temperature (15-19^{o}C) under short photoperiod (12h) was more suitable for early tuber growth.

2.2.3.1.1. Tuberization of whole plants

Tuber induction is promoted by short photoperiods (or, more accurately, by long nights) and the signal is perceived in the leaves (Gregory 1965. Cited by Ewing and Struik, 1992). Phytochrome is apparently involved in perceiving the signal: tuberization was diminished when long nights were interrupted by red light, and this effect was partially reversed by subsequent exposure to far-red light. Weeler and Tibbitts 1986, (cited by Ewing and Struik, 1992) have reported that light quality also played an important role in tuberization. Applying the long photoperiods at later stages of growth resulted in the delayed onset of tuber growth, and tuber bulking occurred probably more at higher than at lower temperatures. Extension of the photoperiod was the most effective treatment for delaying tuberization, and doubling the final yield from transplanted seedlings (Engels *et al.*, 1995). The effect of the photoperiod on the period between onset of tuber growth and tuber bulking is probably relevant for the number of tubers, reaching harvestable size, and source sink relations during the growing period (Vandam *et al.*, 1996). The number of tubers depended on the number of potential tuber sites and on the relative set which was surprisingly high for those treatments in which a high stolon temperature compared with a low root temperature(Struik *et al.*, 1989). In the whole plant, tuberization is greatly favored by darkness around the growing point Although tuberisation on cuttings reflects the degree of tuber induction of whole plants too, it is important to point out that it is not perfectly correlated with whether or not tubers are present on the plants from which cuttings were taken (Ewing, 1985).

2.2.3.1.2. Tuberization *in vitro*

Red/ far red light affects the tuberization of *in vitro* potato plants. The application of five minutes of red light to potato sprouts immediately after their excision from the mother tuber accelerated *in vitro* tuberization when. Sprouts were incubated in the dark on a medium which contained 4% glucose (Blanc, 1981. Cited by Ewing and Struik, 1992). The acceleration was reversed by exposure to far red light after the red light. Sensitivity to red

light, far-red light was optimal at the time of excision and disappeared by 6 hours after excision (Ewing and Struik, 1992).

The darkening of both roots and shoots strongly promoted tuber formation *in vitro* culture; the tubers were formed on that part of the plant which was darkened (Aksenova *et al.*, 1994). Application of IAA in the culture medium had no pronounced effect of plant morphogenesis, but slightly increased tuber formation in blue light. In red light IAA significantly changed the induction of tuber formation. Added Kinetin in the culture medium in Red light increased the induction of tuber formation, but these effects were much weaker than that of IAA, but in blue light Kinetin increased the fresh weight of roots and stolons (Aksenova *et al.*, 1994).

2.2.3.2. Temperature

Potato (*Solanum tuberosum* L.) plants form tubers on stolons when conditions for the formation of both stolons and tubers are favourable. The induction, initiation, growth, and subsequent inhibition of elongation of stolons are crucial steps in the tuberization process of potato plants (Vreugdenhil and Struik, 1989). This process is strongly influenced by temperature and photoperiod (Struik *et al.*, 1989a). High temperature delays, impedes or even inhibits the tuber initiation and affects the distribution of dry matter between tuber and haulm. Night temperature, especially, is crucial (Struik *et al.*, 1989a).

High air temperature stimulates the development of stolons and also favours stolon branching. Temperature not only affects the tuberization stimulus in the stolon tip, but it also affects dry matter partitioning to the below-ground plant parts, and the net amount of photosynthate available for the whole plant (Ewing, 1985). The negative effects of high temperature on tuberization are much more pronounced under long photoperiods (Wheeler *et al.* 1986, cited by Ewing and Struik, 1992). The tuber initiation and tuber enlargement exhibited different responses to the temperature condition during plant growth. Tuber formation increased in alternate 25 ^{0}C day and 15 ^{0}C night conditions with short or long daylengths. Tuber formation does not occur in plots subject to long days and a constant

25^0C (Chang and Chang, 1990). Snyder and Ewing (1989) have reported that raising the temperature under a short photoperiod decreased the tuber dry weight of cuttings of the late-maturing Katahdin by about 50%. Long photoperiod exacerbated the negative effect of higher temperature on tuber induction, especially in the later-maturing cultivars Katahdin and Désirée.

High stolon temperature stimulates haulm senescence. It also affected the occurrence of bacterial wilt (*Pseudomonas solanacearum)*, which attacked the plants mainly when the temperature around the stem base was above 28^0C (Struik *et al.,* 1989a). High air temperature does not affect the haulm but will affect the above ground parts of the plants. When air temperature and root temperature were low, high stolon temperature increased the stem yields, but when air and root temperature were high, the effects are always negative (Struik *et al.,* 1989b). Increasing the temperature of the stolon environment greatly increased the number of tubers. This effect depends on the cultivar (Struik *et al.,* 1989c). However, increasing the stolon temperature when either the air temperature or the root temperature was already high caused a large yield reduction (Struik *et al.,* 1989c).

High temperatures are associated with high levels of gibberellins. Gibberellins stimulate starch hydrolysis or inhibit starch synthesis (Smith and Palmer, 1970,; Menzel, 1980.; Struik *et al.,* 1989b). Gibberelins have been shown to promote stolon formation and stimulate stolon elongation by delaying the tuber formation and increasing the rate of growth (Hammes and Nel 1975). All increases in temperature caused complex effects on stolon development and reduced synchronisation of their initiation. The apical dominance of the shoot apex might have been very large, but the high stolon temperature may have caused one stolon to become much more dominant than another (Struik *et al.*, 1989b) The high low temperature pattern is more important for tuber sizing. High temperatures prolonged the period between the onset of tuber growth and bulking. At high temperatures, the lack of sink strength caused by the malfunctioning of starch synthesising enzymes would enhance the formation of many tuber initials, without allowing them to grow to substantial size (Vandam *et al.*, 1996).

Vandam *et al.,* (1996) observed that a delay occurred in the onset of tuber growth with temperatures 15 – 27 ^{0}C. Temperatures 15 – 19 ^{0}C are optimal for tuber initiation. Higher temperatures also delayed the onset of tuber bulking and decreased the absolute tuber growth rate. The delay in the onset of tuber growth tended to be smaller than the delay in the onset of tuber growth and bulking Vandam *et al.,* (1996). This effect may enhance the formation of competitive tubers in the lower part of the temperature range, especially at high levels of radiation. At high temperatures, the lack of sink strength caused by the malfunctioning of starch synthesis enzymes would enhance the formation of many tuber initials, without allowing them to grow to substantial size (Vandam *et al.,* 1996). High temperatures of the shoots, and to a lesser extent of the roots, delayed tuber initiation. Stolon growth was stimulated moderately whether temperatures of shoots, roots or stolons and tubers were raised (Struik *et al.*, 1989b). It is reported that, when the air temperature is high , it is possible to obtain acceptable yields of good quality tubers.

2.2.3.3. Irradiance

Like long photoperiods and high temperatures, low levels of irradiance during the day decrease the induction required for tuberization (Bodlaender 1963) cited by Ewing and Struik, 1992. It is suggested that the effects of both low light intensity and high temperature are brought about by increased production of growth substances which inhibit tuber formation. Gibberellins are the most likely candidates for such role. As might be expected, a combination of high temperatures and low irradiance is especially inhibitory to tuberization (Menzel 1985).

2.2.3.4. Nitrogen and pH

A fourth environmental factor which plays a major role in induction to tuberize is N fertilisation. In their classical work on tuberization, Ewing and Struik, (1992) conclude that limiting the supply of inorganic N to plants which are growing vigorously because of exposure to long days and warm temperatures produces a retardation of shoot growth. N application increased both stolon numbers and their growth period. During tuber initiation,

there was a period of stagnation in stolon growth; N application extended this period, but this depended on cultivars. The tuberization of cuttings from late maturing cultivars was decreased by high rates of N (McGrady and Ewing 1990).
The higher N rate retarded tuber initiation early in the growing season. It was not considered possible to correlate tuber yield with rate of stolon development or stolon weight (Zrust and Mica 1992). This was accompanied by the accumulation of starch in the shoots, and with initiation or acceleration of tuberization.

Under field conditions the effect of N application on delaying the time of tuber initiation was also small (Radley 1963) cited by Ewing and Struik, (1992). The tuber initiation delay would be more obvious under tropical conditions where warm temperature would tend to discourage tuberization (Ewing and Struik, 1992). The growth of potato plants was inadequate in NH_4 nutrient compared to NO_3. Under field conditions, as the stolon number, especially the number of 2^{nd} order stolons, increased in the NO_3 N applied plot, it was assumed that NO_3 N promoted the branching of stolons and stolon growth, but tuber growth was accelerated by NH_4 N (Osaki *et al.*, 1995).

Hydroponically growing plants of the *tuberosum* cultivars did not tuberize under 12-h photoperiods in this system unless the N supply was temporarily withdrawn. In solution culture N deficiency promoted tuberization, but tuberization was not induced by N withdrawal under 18-h photoperiods or under constant temperatures of 30^0C (Krauss and Marschner 1982). In mist culture N deficiency and interruption of mist for 12 h induced rapid tuberization (Wan *et al.*, 1994). In water culture plant growth was reduced by NH4-N compared to NO_3 N (Polizotto et al., 1975). NH_4 N was a good nitrogen source for vegetative growth, while NO_3 N was suitable for reproductive growth.

In water culture, tuber initiation was induced in the plants subjected to intermittent pH reductions compared to constant pH 5.5; the induction was greatest with the lowest pH. The pH 4.0 is close to critical low pH for effective tuber induction in potatoes. Short term reduction of solution pH, can significantly promote tuber initiation on potato plants grown

with stolons immersed in liquid, for both rapid and consistent tuber induction, the intermittently pH levels need to be <4.0 (Wan *et al.*, 1994).

2.2.4. Hormonal factors

The mechanism of stolon and tuber formation can be affected somewhat differently by different environmental conditions and by different hormonal treatments. Stolon initiation can occur even before the leafy shoot has emerged, so it does not depend on signals from the shoots. Chapman (1958) reported the occurrence of a specific tuberization stimulus which is formed in the leaves under the short days and transmitted to underground part to induce tuberization. So tuberization is dependent upon signals from the shoots.

2.2.4.1. Cytokinin, ABA and IAA

Some researchers have speculated that the stimulus may be similar to cytokinin or ABA. However (Koda and Okazawa 1983) cited by Koda and Okazawa (1988) suggested that, although cytokinin and ABA have some roles in the tuberisation process, they are not the tuberization stimulus which trigger the process. For *in vitro* potato plants, exogenous application of cytokinin may play an important, but not a key-role in the micro-tuberization process (Galis *et al.*, 1995). Sergeeva *et al.*, (1994) reported that *in vitro* culture under all conditions where tubers were formed, there was also a relatively high level of cytokinin in the roots. Kinetin in the medium in blue light changed the distribution of cytokinin and significantly increased the IAA level in roots. In red light, the presence of kinetin led to an increased cytokinins level in the whole plant, while the IAA level was slightly lower. A high level of IAA in roots may increase the percentage of sucrose remaining therein, may increase assimilates going to roots, and thus stimulate tuberization.

2.2.4.2. Jasmonic acid:

Koda and Okazawa (1988) found two acidic substances which are active in inducing potato tuberization. The activity of tuber-inducing substance in leaves continued to increase under the tuber-inducing condition, but remained almost constant under the non- inducing condition. These substances cause the cessation of stolon elongation and induce slight swelling at the subapical region of the stolon. In their experiments, cytokinin and ABA failed to induce the tuberization, but cytokinin and ABA accumulate in the swelling part. Pelacho and Mingo-Castel (1991) found the tuber-inducing substance closely related to jasmonic acid (JA). It can be assumed that jasmonic acid or a related compound is the most active factor in tuber induction. Jasmonic acid would act as a chemical signal to trigger senescence- related processes, like tuberization, which take place after a sufficient vegetative development of the potato plant.

Jackson and Willmitzer (1994) found jasmonic acid is not a tuber-inducing signal transported from the leaves, because long photoperiod and night breaks either have an inhibitory effect on tuberization or, alternatively, are conditions in which the tuberisation signal is not produced in the leaves or, when produced, is not transported out of the leaves. In either case, the effect of long days or a night break does not appear to be either overcome or complemented by the spraying of jasmonic acid. This result would argue against jasmonic acid being the transported tuber-inducing signal. Jackson and Willmitzer (1994) suggest that jasmonic acid may play some role in tuber formation after induction has taken place. For potato tuber induction a short day and low temperature are important.

Jasmonic acid in foliage, roots, stolons and tuber periderum extracts incorporated into the culture medium and applied by microdrop to the cuttings, showed a lower response in tuberisation percentage than was obtained with endogenous jasmonic acid extracted from potato foliage. Meanwhile, the highest response was observed with tuber periderum extract. A rather lower response was obtained with roots and stolons extracts (Abdala *et al.*,1996). Yoshihara *et al.,* (1996) who reported that, when radioactive lablled jasmonic acid (2-^{14}C) was applied to potato plants grown under tuber inducing condition (short day)

and non inducing condition (long day), observed jasmonic acid metabolised into its glucoside (Tuberonic acid glucoside) (TAG) through glucolisation and tuberonic acid glucoside then moved to the underground part of the plants. Under the long day condition production of tubers was not observed; and low radioactivity was present in the stolons. Under the short day condition, tuberisation was found within 10 days after application and radioactivity was present in the stolon and tubers. From this result he suggested that when the concentration of TAG reaches a high enough level, tuberization is induced.

2.2.4.3. Gibbrellic acid

Gibbrellic acid applied to the potato plant has been found to promote growth and branching of stolons, and increase the tuber number of tuber site (Bodlaender and van de Waart, 1989.; Haverkort and Marinus 1995.; Caldiz, 1996) but reduce tuber formation. Under long days the GA effect was magnified (Hammes and Nel 1975). Inhibition of tuberization occurs as a result of exogenous application of gibberellin, spraying the plants with gibberellic acid in concentrations of 10.25 and 50mg/l induced stolon formation in the leaf axils. It led to a decrease in the number of tubers formed per plant (Haverkort and Marinus, 1995, Abdala *et al.,* 1994). Woolley and Wareing (1972) found that environmental conditions which lower induction to tuberise (long day, high temperature, low irradiance, high N fertilisation) were associated with higher levels of gibberellin activity. Multiple harvesting of the largest tubers from plants, treated with gibberellic were approximately double the number of tubers formed. It halved the individual weight compared with only one harvest at plant senescence (Haverkort and Marinus, 1995).

2.2.4.4. Ethylene

Ethylene production in plant tissue occur in different situation and different quantity. Ethylene production in plant tissues increases upon various type of stress (Vreugdenhil and Struik, 1989). Potato stolons produce ethylene upon mechanical constraint in the soil. As result, stolon elongation will stop, provided that level of gibberellins is sufficiently low (Vreugdenhil and Dijk, 1989). Lugt *et al.*, (1964) reported extremely vigorous stolon

growth and a delay of tuberization when the stolon environment did not provide enough mechanical resistance or low level of ethylene in stolon.

2.2.5. Carbohydrate

The process of tuberisation comprises inhibition of the longitudinal growth of the stolon followed by the initiation and growth of the tuber. During tuberisation, two major biochemical changes occur, normally accumulation of starch and the formation of storage proteins (Visser *et al.*, 1994). At one time the level of non-structural carbohydrate in the leaf was believed to be the controlling factor in the induction of tuberisation. According to this hypothesis, short photoperiods and cool temperatures slow down leaf growth causing the accumulation of assimilate and a high C:N ratio, which in turn brings about tuberisation (Garner and Allard, 1923, Wellensiek, 1929, Werner, 1934) Cited by Ewing and Struik, (1992). It is possible that starch accumulation in the leaves plays a significant role in tuber induction. The maximum starch concentrations in leaves were obtained in the high light (600 µmol.m-2.s-1 photosynthetic photon flux) treatment at short photoperiods rather than in the low light (300 µmol.m-2.s-1 photosynthetic photon flux) treatments (Stutte *et al.*, 1996). It is generally accepted that long photoperiods inhibit tuber initiation and promote potato shoot growth.

In addition, there are significant changes in the content and composition of the soluble sugar pool in tuberising stolon tips (Davies, 1984; Ross et al., 1994).
Sucrose may enhance tuber formation. In intact potato plants, assimilates may provide part of the stimuli for tuber formation (Ewing and Struik, 1992). It was reported that tuberisation is negatively correlated with the level of reducing sugars (mostly glucose) (Darpas *et al.*, 1986; Hawker *et al.*, 1979) cited by Simko (1994), but in his experiments he found that no significant correlation existed between glucose level and percentage of tuberised segments. Furthermore, a positive correlation existed between the level of glucose and the weight of micro-tubers.

The tuber induction process is primarily regulated by a balance between gibberellins and promoting substance (Okazawa and Chapman, 1962). The present hypothesis is that a high exogenous sucrose supply causes the formation of excess UDPglucose, which in turn increases the conjugation of free GA. In this way the sucrose flux would directly change the level of active hormones participating in potato tuber induction. A simplified scheme to show a sucrose role in potato tuberization via the formation of glucose conjugates.

According to this hypothesis, glucose does not significantly affect potato tuberization, because only a small amount of endogenous glucose is converted to sucrose. Tuber induction in potato does not require a high level of endogenous sucrose. However, the high sucrose apparently provides a favourable environment in which the tuberization stimulus can act (Simko, 1994). At tuberization the activity of soluble acid invertase decreased, and that of sucrose synthetase increased. Under the non-tuberizing condition the activity of both enzymes remained unchanged. The activity of fructokinase and hexokinase both increased after tuber initiation, but remained unchanged in the non tuberizing condition. The increase in total glucose-phosphorylating potential could be a response to the tuberization-related starch accumulation process (Appeldoorn *et al.*, 1997).

Chapter Three

General materials and methods

3.1. Experimental site

The experiments were carried out from July 1997 to June 1998 at the Plant Growth Unit (PGU), Massey University, Palmerston North, New Zealand (Lat. 40 ° 23 S, Long.175° 37 E). Six experiments were conducted.

3.2. Growing conditions

Large containers (20 litre) were used to grow the plants in deep flow, aeroponics and bark systems. The NFT system used a wooden channel with the internal dimensions of 180 cm x 25 cm x 10 cm covered by double-sided (black and white) polythene sheet (Panda film). The white side was placed outwards to reflect the light. The top of the channel was covered with Panda film and plastic wire mesh. The NFT channel was sloped to the same level water (0.5 cm) in the system. The nutrient solution was recycled continuously at a rate of 500 ml/minute.

The 20 litre container's outer surface was first painted black to exclude light, and painted white to minimise solution heating. The aeroponics system comprised two irrigation nozzles fixed inside the container. The nutrient solution continuous sprayed onto the root zone. Each container was checked daily to ensure that it was working.

In the deep flow system nutrient solution was supplied continuous to each container at a rate of 300 ml/ minute through the tube at the bottom of the container and drained out from a tube at the top of the container to maintain the solution depth at 30 cm. The main tank nutrient solution and deep flow container solutions were aerated with an air diffuser at the bottom of the container.

The solution in the main tank was maintained at a constant level with an automatic float valve which controlled the level of the solution. The pH was maintained at a range between 5.5 to 6.5. The conductivity of the solution was maintained at 20CF. The solution pH and conductivity were monitored daily and adjusted if needed. The conductivity level was measured using a CF meter and the pH by a pH meter. The nutrient solution was replaced at the end of every experiment when the whole system was disinfected with 5% sodium hypochlorite solution.

The 'bark system' containers were filled with 100% bark medium to a depth of 30 cm. An automatic feeder was used to supply the nutrient solution four times daily at a rate of 75 ml/ minute for 10 minutes.

A 400 litre nutrient solution tank was placed below ground level with the top of the tank lower than the drainage line. The tank was covered to exclude light and reduce contamination. The solution was pumped to the solution supply line at a rate of approximately 3 litres per minute by a Mono water pump, model CP25. The solution then flowed down the drainage line and back to the tank.

The glasshouse temperature was controlled by heating when the temperature was less than 15 °C, and by ventilating when the temperature reached 25°C.

3.3. Planting materials

Four potato cultivars, Russet Burbank (RB), Kennebec (K), Atlantic (A), and Désirée (D) were used in the experiments. Virus free *in vitro* plantlets were obtained from a tissue culture laboratory in Christchurch and used as the initial planting material.

3.4. Treatment and experimental design

A split plot design with four replicates was used for this experiment. The treatments consisted of four different production systems (bark, aeroponics, deep flow and NFT) as

main plots and four potato cultivars Russet Burbank (RB), Kennebec (K), Atlantic (A), and Désirée (D) as the split plots. The spacing between plants was 15 cm, and the space between each plot was 75cm. Guard plants were grown around the glasshouse.

3.5. Nutrient solutions

3.5.1. Nutrient solutions for hydroponics systems

For the hydroponics systems, the stock solutions were prepared and stored in four separate containers.

Table 3.1 The four stock solutions

Chemicals		Per 20 lt
Name	Formula	
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	1.976 kg
Potassium nitrate	KNO_3	1.316 kg
Mono potassium phosphate	KH_2PO_4	0.544kg
Magnesium sulphate	$MgSO_4.7H_2O$	0.993 kg
Chelate iron	$FeNa_2$ EDTA	0.158 kg
Manganous sulphate	$MnSO_4.H_2O$	12.3g
Boric acid	H_3BO_3	3.42g
Copper sulphate	$CuSO_4.5H_2O$	0.55g
Ammonium molybdate	$(NH4)_6Mo_7O_{24}.4H_2O$	0.184g
Zinc sulphate	$ZnSO_4.7H_2O$	0.62g

(Plant Growth Unit Massey University)

The bark system nutrient solutions (A & B) were prepared and stored in two separate containers as recommended by Tregidga *et al.*, (1986) for cucumbers and tomatoes grown in pumice.

Table 3.2. The bark system nutrient solutions (A & B)

Chemicals		Per 100 lt
Name	Formula	
Calcium nitrate	$Ca(NO_3)_2.4H_2O$	9.88 kg
Chelate iron	$FeNa_2$	0.789 kg
Potassium nitrate	KNO_3	6.581 kg
Mono potassium phosphate	KH_2PO_4	2.72 kg
Magnesium sulphate	$MgSO_4.7H_2O$	4.966 kg
Manganous sulphate	$MnSO_4.H_2O$	61.54g
Boric acid	H_3BO_3	17.14g
Copper sulphate	$CuSO_4.5H_2O$	2.75g
Ammonium molybdate	$(NH4)_6Mo_7O_{24}.4H_2O$	0.92g
Zinc sulphate	$ZnSO_4.7H_2O$	3.08g

3.6. Crop management

White fly and Aphids were the only pests observed at any time. Insecticide sprays were used in the glasshouse when necessary. In the winter no diseases occurred on the plants, but during the summer bacterial rot was observed on plants which were grown in all systems except bark media. Ozone was injected into the nutrient solution, but did not control the root and stem rotting. (Hort Ozone Horticulture water treatment, model- ADI was used).

3.7. Data collection

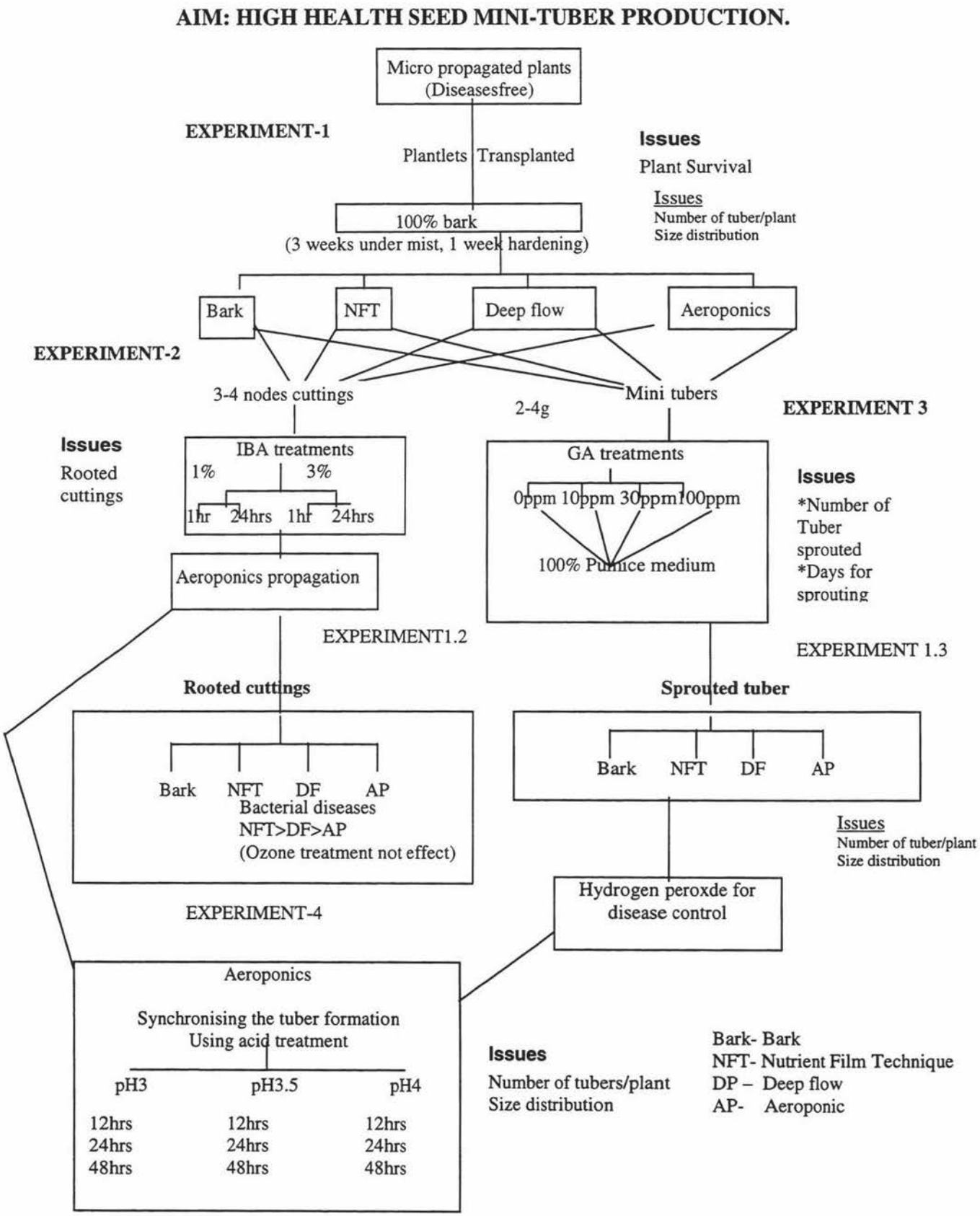
Plants were harvested 12 weeks after transplanting into there final layout. The following data were recorded.

a. Number of tubers per plant
b. Stolon fresh weight per plant
c. Stolon dry weight per plant
d. Average stolon diameter per plant
e. Largest tuber weight
f. Average tuber weight
g. Tuber size distribution.

3.8. Analysis of data

Analysis of variance (ANOVA) was performed using Statistical Analysis Systems (SAS) software Version 6.12. Comparisons of means were carried out using the Duncan's multiple range test at 5% level of significance.

3.9. Outline of experiments



Chapter four

Comparison of minituber production systems with different Potato cultivars

4.1. Experiment –1 Minituber production using *in vitro* plantlets

4.1.1. Introduction

There are different kinds of production systems used in the world for the intensive production of vegetable and ornamental crops. The use of different systems depends on the knowledge and economic condition of the people. In recent years the usage of hydroponics systems has been increased considerably. These system seem to eliminate soil borne pests and diseases, and minimise water and nutrient losses. In this experiment, four different systems were used to evaluate minituber production with different cultivars (Russet Burbank, Kennebec, Atlantic, and Désirée).

1. Bark - Bark
2. Deep flow - DF
3. Nutrients Film Technique - NFT
4. Aeroponic - AP

The first generation propagules of seed potatoes (minituber) are high economic value. Direct transplanting of whole *in vitro* plantlets at high density in porous substrates such as bark, peat or mixtures of peat with an inert substrate usually multiply them in greenhouse. Replacing such substrates with more expensive hydroponic systems may be economically viable if increased production. A high humidity chamber and intermittent misting ensured that 95% of plants survived for tuber production (Vodenik, 1990). Large quantities of mini-tubers can be produced from *in vitro* plantlets by repeated harvesting after 4, 7, and 10 weeks after transplanting (Ranalli *et al.*, 1994). Therefore, comparison of production systems, by using *in vitro* plantlets is useful for minituber production.

Mini-tubers are small seed potato tubers which are produced by repeated harvesting in the glasshouse on plantlets propagated *in vitro*. Producing high number of minitubers can reduce the number of field multiplications required for the production of high health seed potatoes. Under New Zealand's climatic conditions, field planting is possible only once a year, so seed tuber bulking normally takes several years for commercial production.

The object of this experiment was

1) to compare the production systems for production of seed potatoes from *in vitro* plants; and
2) to compare the production systems for production of seed potatoes from different cultivars.

4.1.2. Materials and methods

All growing conditions and methods are the same as described in Chapter Three.

Plant material

As in explain Chapter Three, disease- free *in vitro* plantlets of cultivars Russet Burbank, Kenebec, Atlantic, and Désirée were obtained from a tissue culture laboratory in Christchurch, New Zealand and used in the study.

Transplant the potato *in vitro* plants in different systems.

The four week old *in vitro* plantlets were washed in warm water and separated from agar medium. Separated plantlets were kept in water to prevent water loss during the transplanting time. Plantlets were set initially in a pure bark medium and kept in an intermittent mist propagation tent in the Plant Growth Unit at Massey University.

When the plantlets had recovered from transplant shock and started growing as normal plants, they were taken out of the mist system and hardened off. After 4 weeks the

established plants were washed to remove the bark particles from the roots and planted in the bark, deep flow, NFT and aeroponic systems. Plants are randomly allocated to each treatment. Tetron wool was used to hold the plants in aeroponics and deep flow systems.

Three plantlets were put separate holes in each container. Holes were made in triangle pattern with about 15 cm between two holes for aeroponics and hydroponics systems. For the bark system 3 plantlets were planted in each container in the medium about 15 cm apart in a triangular pattern. In the NFT system plantlets were planted with 15 cm between plants.

4.1.3. Results

Table 4.1.1. Effect of production system on tubers per plant, yield per plant, Largest tuber weight, average tuber weight and stolon diameter

System	No of tubers per plant	Largest tuber weight (g)	Average tuber Wt (g)	Yield per plant (g)	Stolon diameter (mm)
Bark	15.40 b	109.23 a	12.15 a	190.11 a	1.54 b
DF	29.60 ab	21.19 b	2.07 b	57.89 b	1.98a b
NFT	15.31b	27.76 b	3.66 b	56.96 b	2.17 a
AP	37.34 a	32.78 b	2.45 b	82.57 b	2.42 a

Means with same letter are not significantly different at 5% level.

When using tissue cultured plants as the initial planting materials for mini-tuber production in different systems, the tuber yield per plant and largest tuber weight were significantly higher in the bark system than in the other systems (Table-4.1.1) The aeroponics system produced more tubers than other systems with an average weight of 2.45 grams. Aeroponics and NFT systems produced thicker stolons when compared with the bark and deep flow systems.

Table 4.1.2. Effect of cultivar on tubers per plant, yield per plant, largest tuber weight, and average tuber weight and stolon diameter.

Cultivar	Tubers per plant	Largest tuber weight (g)	Average tuber Wt (g)	Yield per plant (g)	Stolon (mm) diameter
Russet Burbank	15.71 a	51.66 ab	4.98 a	97.27 a	1.83 bc
Kenebec	18.41 a	71.38 a	5.75 a	99.48 a	2.64 a
Atlantic	33.12 a	36.26 b	5.02 a	101.67 a	1.50 c
Désirée	29.56 a	29.97 b	4.30 a	85.78 a	2.15 ab

Means with same letter are not significantly different at 5% level.

These results show that there was no significant difference in number of tubers per plant, but cultivar Kenebec produced higher weight (71.38g) tubers than cultivar Atlantic and Désirée. Average tuber weight, and yield per plant, were not significantly different. The variety Kenebec produced thicker stolons (2.64 mm) than other cultivars, but cultivar Atlantic produced thinner stolons (1.5mm).

Table 4.1.3. Percent tuber size distribution in each production systems

System	1 – 2g	3 – 4g	5 – 9g	10 – 24g	>25g
Bark	30.15c	16.67a	17.91a	22.22a	14.35a
DF	72.95ab	25.59a	6.43b	2.811c	0.80a
NFT	51.90bc	25.12a	12.77ab	12.05b	2.14a
AP	82.43a	16.75a	8.39b	3.84c	0.89a

Means with same letter are not significantly different at 5% level

Table 4.1.4. Percent tuber size distribution in different cultivars.

Cultivar	1 – 2g	3 – 4g	5 – 9g	10 – 24g	>25g
Russet Burbank	65.27 a	21.11 a	9.70 a	9.10 a	4.00 b
Kenebec	56.28 a	18.91 a	13.67 a	9.50 a	6.80 a
Atlantic	54.15 a	21.67 a	11.83 a	13.50 a	3.90 b
Désirée	63.23 a	21.77 a	10.22 a	8.50 a	3.00 b

Means with same letter are not significantly different at 5% level

These results show that the aeroponics system produced a higher percentage (82.43%) of 1-2g size tubers than the other systems. The bark system produced a much higher percentage (36.57 %) tubers > 10g size than did other systems (Table- 4.1.3). Tuber size distribution was not affected by cultivar differences (Table- 4.1.4).

4.1.4. Discussion

In this experiment, stolons grew continuously in hydroponics systems, because there was no mechanical resistance to stop longitudinal stolon growth. The degree of mechanical resistance encountered by the extending stolon may affect the stolon and tuber development (Vreugdenhil and Struik, 1989). In the hydroponics systems, because developing stolons did not encounter sufficient mechanical resistance they had extremely vigorous growth. This tends to provide more potential sites for tubers. Longitudinal growth of a stolon will continues as long as condition are unfavourable for tuberization. Rapid growing stolons produced tubers continually when environmental conditions favoured tuberization. Long day (LD) conditions favour stolon elongation, were as short days (SD) result in cessation of stolon growth (Chapman 1958).

High air temperature stimulated the development of stolons and also favoured for stolon branching (Ewing, 1985; Struik *et al.,* 1989b; Ewing and Struik, 1992). Temperature not only affects the tuberization stimulus in the stolon tip, but it also affects dry matter partitioning to the below-ground plant parts, and the net amount of photosynthate available for the whole plant (Ewing, 1985). The negative effects of high temperature on tuberization are much more pronounced under long photoperiods (Wheeler *et al.*, 1986, cited by Ewing and Struik, 1992). Therefore maximum tuber production requires an optimal temperature- photoperiod combination. Vandam *et al.,* (1996) concluded that a low mean temperature (15-19°C) under short photoperiod (12h) was more suitable for early tuber growth.

Gibberellins stimulate starch hydrolysis or inhibit starch synthesis (Smith and Palmer, 1970; Menzel, 1980; Struik *et al.,* 1989b). Gibberellins have been shown to promote stolon formation and stimulate stolon elongation by delaying the tuber formation and increasing

the rate of growth (Hammes and Nel 1975). Vreugdenhil and Struik, (1989) have reported that ethylene and gibberellins influence tuber formation, but only in a negative way. Ethylene production in plant tissues increases upon various type of stress (Vreugdenhil and Struik, 1989). It was suggested by Vreugdenhil and Struik, (1989) that elongation of stolons is controlled by an antagonism between gibberellins and ethylene, the level of gibberellins decreasing under tuber-inducing conditions, whereas the production of ethylene in the stolon might increase temporally as result of mechanical resistance of the soils.

At an early stage of plant development we observed that, although many stolons formed on the stem, they did not tuberize in deep flow and aeroponic system under short days, but tuber formed in NFT and bark based systems. This could be lack of mechanical resistance, related with high level of gibberellins and low level of ethylene in stolons. Tuber initiation in potatoes occurs readily in solid matrix culture (Tibbitts and Cao, 1994; Tibbitts and wheeler, 1987) and efficiently with NFT (Wheeler *et al.*, 1990). Tibbitts and Cao, (1994) have reported that, tuberization is inhibited when roots and stolons are immersed in solution or continue mist culture.

At the late stage of plant development, tubers were formed on stolons at the time the potato plants were fully developed and covered the all the spaces, roots and stolon fill the containers. Wan *et al.,* (1994) have reported same observation, tuberization does occur in solution culture when the container becomes filled with roots and stolons. This observation could suggest that when hydroponics systems are used for tuber production, they need more mechanical resistance or some other alternative way to stop the stolons' growth. Gregory, (1965) has reported that tuber induction is under photoperiod control, and the stimulus is perceived in the leaves. When plants are fully developed, more leaf area receives photoperiod stimulus or high level of non-structural carbohydrate in the leaf. It was believed to be the controlling factor in the induction of tuberisation. According to this hypothesis, short photoperiods and cool temperatures slow down leaf growth causing the accumulation of assimilate and a high C:N ratio, which in turn brings about tuberisation (Garner and Allard, 1923.; Wellensiek, 1929,; Werner, 1934) cited by Ewing and Struik,

(1992). The activity of tuber inducing substances in leaves continued to increase under the tuber inducing condition (Koda and Okazawa, 1988). Visser *et al.,* (1994) has reported that the process of tuberisation comprises inhibition of the longitudinal growth of the stolon followed by the initiation and growth of the tuber. It is possible that starch accumulation in the leaves plays a significant role in tuber induction..

Photoperiod interacts with other factors such as temperature, mother tuber physiological age, N nutrients and endogenous hormonal factors for tuberization. Short day (SD) promotes tuberization while long day (LD) or continuous light delays this phenomenon. Chapman (1958) reported the possible occurrence of a specific tuberization stimulus which is formed in the leaves under the short days and was transmitted to the underground part to induce tuberization.

It was observed in this experiment, yield per plant, average tuber weight, and largest tuber weight were higher for in the bark based system because there was enough mechanical resistance to control the stolon growth and tuberization. Ethylene production in the stolons increase temporarily as a result of mechanical constraint in the soil and play an important role in the early steps of tuberization in potatoes by blocking elongation of the stolons (Vreugdenhil and Dijk, 1989). Lagt et al., (1964) have reported extremely vigorous stolon growth and a delayed tuberization when the stolons did not get enough mechanical resistance. It was observed that hydroponics systems produced thicker (1.92-2.42 mm) and longer stolons than bark systems. So hydroponics grown potato stolons need mechanical resistance to tuberized.

When the tuber size distribution was considered, the hydroponics systems produced a higher percentage of 1-2 g size tubers than the bark system. These results clearly indicate that tubers are formed continuous in hydroponics systems. Which may be related to continuous development of stolons in hydroponics systems. Gray (1973) stated that a removal of the mechanical resistance in an early stage of plant development induced the formation of secondary stolons and numerous small tubers. This statement support the tuberization pattern in aeroponic and deep flow systems. Within the hydroponics systems

the NFT system produced a lower percentage of 1-2 g size tubers (51.9%) at the time of harvesting. Tuber initiation in potatoes occurs readily in solid matrix culture (Tibbitts and Cao, 1994; Tibbitts and wheeler, 1987) and efficiently with NFT (Wheeler *et al.*, 1990). In aeroponic and deep flow systems, stolons were freely hanging in buckets, but in NFT systems, stolons grew on the NFT channel surface. NFT channel surface give some resistance on stolons. Therefore NFT system grown potato plants tuberized earlier than deep flow and aeroponic systems grown potato plants.

The variation is caused partly by stolon characteristics, including their date of initiation, position and size. At any given time the tubers on one stem vary in size. The differences in size are partly related to differences in the date of tuber set, and in the rate of growth. However, it is commonly believed that the rate of growth of each individual tuber fluctuates (Gray, 1973; Ahmed and Sagar, 1981; Struik et al., 1988). Ranalli *et al.*, (1994) showed the tuber size distribution was influenced by the source of the planting material the position, date of initiation and size of stolon to which the tuber was attached and variation in external conditions (Struik *et al.*, 1991), but the above results indicate that production methods also affect the tuber size distribution. Aeroponic and deep flow systems produced a higher percentage of smaller tubers than bark and NFT systems.

4.2. Experiment- 2 Minituber production, using rooted cuttings

4.2.1 Introduction

Using good quality potato cuttings is a cheap method of potato production. Propagation of potato seed stocks by stem cuttings was developed in the 1960s as a means of eliminating bacterial and fungal pathogens normally carried over by tuber propagation (Jones, 1991). Multiplication of potato plants by stem cuttings is also a fast method of propagation.

The main objective of this experiment is repeat the first experiment, using rooted cuttings as planting material.

The object of this experiment was

1) to compare the yields of seed potatoes from rooted cuttings in different production systems; and
2) to compare the yields of seed potatoes from different cultivars.

4.2.2. Materials and methods

All growing conditions and methods are the same as described in Chapter Three.

Plant materials.

Primary mother plants, initially raised *in vitro*, were planted in hydroponics systems (as described in the first experiment). Aeroponically propagated apical shoots with 3-4 inter nodes cuttings were used this experiment. Other experimental conditions were the same

Transplant the rooted cuttings in to different systems.

Uniform sized two weeks old rooted cuttings were taken out of the aeroponic propagation system. Plants were randomly allocated to each treatment. Tetron wool was used to hold the plants in the aeroponic and deep flow systems.

Three rooted cuttings were put in separate holes in each container. Holes were made in triangle pattern with about 15 cm between two holes for aeroponics and hydroponics systems. For the bark system, 3 rooted cuttings were planted in each container in the medium about 15 cm apart in a triangular pattern. In the NFT system rooted cuttings were planted with 15 cm between plants as for the first experiments.

Disease control

An ozone generator was used to control the disease problems on root of cuttings. It was used for two hours every two days to control bacterial disease on the root system, and to sterilize the nutrient solution as directed by Jarvis (1992) for inhibiting microbial growth in nutrient solutions.

4.2.3. Result

The experiment was stopped 8 weeks after the transplanting of rooted cuttings. Stem rot was observed on these potato plants, no data was gathered from this experiment, but useful observations were gathered.

Rooted cuttings were well established in different systems and crop cover was 100% after four weeks. Stolons were formed from nodes of the cuttings. The aeroponic system plants produced more stolons than NFT or the deep flow system over a long period. Stem rot was observed on the stem at the interface of water and air 5 to 6 weeks after transplanting. The first affected plant was observed in the NFT system, followed by the aeroponic and deep flow systems. Finally, this disease affected all hydroponic plants. Ozone treatment did not control the disease. No stem rot was observed in the bark systems.

Disease identification

Samples were collected from affected plants. Stems were crushed in sterile water and incubated onto freshly cut potato slices in a humid container at 24 °C for 24 – 48 hours. They developed a definite blackening of the tissue, but the smell and softening of the tissue typical of *Erwinia* infection did not occur.

Infected potato slices were plated onto crystal violet pectate agar to promote spore growth.

No liquefaction of the media occurred, indicating *Erwinia* was not present. When infected potato slices were plated on to King's medium, abundant growth and a strong fluorescence indicated a *Pseudomonas spp.* was present.

4.2.4. Discussion

Experiment was conducted during the summer (December to February). Due to the long days and high sunshine hours, higher temperatures (28 – 30 ^{0}C) than ambient (18 – 20 ^{0}C) occurred inside the glasshouse. The glasshouse was passively cooled to control temperature, side and roof ventilators could open and promote air circulattion. Higher glasshouse temperatures increased the solution temperature by 8 to 10 ^{0}C in the buckets and NFT channels when compared with the first experiments. High temperature and moisture provide an ideal environment for some pathogens proliferation (Favrin *et al*, 1988). Stem rot was first observed in the NFT system and this spread to other hydroponics systems through the nutrient solution, as only one tank was used to store the nutrient solution for all hydroponics systems. Re-circulation of the nutrient solution could easily spread pathogens entering the storage tank (Zinnen, 1988). We identified the organism which caused the stem rot as a *Pseudomonas spp.* Struik *et al.*, (1989a) have reported high temperature affected the occurrence of bacterial wilt *(Pseudomonas solanacearum),* which attacked the plants when the temperature around the stem base was above 28^{0}C.

Pseudomonas solanaceraum has been reported as a pathogen in tomato soil-less culture (Gullino and Garibaldi, 1994). *Pseudomonas* inhibiting plant growth can easily develop in hydroponic systems (van Peer *et al.*, 1990). In traditional cultivation methods, some pathogens considered only minor pathogens may, in hydroculture system, develop into major pathogens.

A potential possible explanation of why the stem rot was observed first in the NFT system because of high temperatures in NFT solution as there was only a thin layer of solution in NFT channels, and slow movement of solution in the channels. Favrin *et al.*, (1988) reported that high temperature and moisture provide ideal conditions for most of the pathogens.

The movement from growing plants in soil to soil less cultures has not produced a situation where soil-borne diseases on completely controlled. Most root-infecting pathogens also occur in the soil-less growing new cultivation systems (Runia, 1994). Berkelmann *et al.*, (1994) have reported that *Pseudomonas* was detectable in high densities and seemed to play a dominant role to produced the disease in the case of hydroponics with recycled nutrient solution. Their optimal growth temperature and their strictly aerobic metabolism fits well into, nutrient solutions offer which ideal conditions for extensive multiplication of *Pseudomonas* in a nitrogen-rich, and well oxygenated environment.

4.3. Experiments: 3 Minituber production using minituber

4.3.1. Introduction

Potato minitubers can be used as a propagation material for the production of high quality seed in a seed programme. The average weight of the minitubers, depends on the technique used for their production (Lommen and Struik, 1995).Tuber multiplication factors may be affected by production systems. Multiplication factors can be expressed in terms of the number or weight of progeny tubers produced per tuber or per unit weight.

The main objective of this experiment was to repeat the first experiment, using minitubers as planting materials.

The object of this experiment was
1. to compare the yields of seed potatoes from rooted cuttings in different systems; and
2. to compare the yields of seed potatoes from different cultivars.

4.3.2. Materials and methods

All growing conditions and methods were the same as described in Chapter Three.

Plant materials

Aeroponic, Deep flow and NFT grown *in vitro* plants produced minitubers were stored at 5°C for two months. In this experiment, minitubers (2-4g weight) were selected, treated with 30 ppm GA for 10 minutes to break the dormancy and sprouted in 100% pumice medium at room temperature. Sprouted tubers were then transplanted into the same system used in the first and second experiments.

Diseases Management

Hydrogen peroxide was used in the nutrient solution to prevent the outbreak of disease. 3 – 10 ppm of active ingredient of hydrogen peroxide was maintained throughout the experiment.

4.3.3. Results

Table 4.3.1. Effect of Production system on tubers per plant, and yield per plant

System	Tubers per plant	Yield per plant (g)
Bark	5.3b	191.1a
DF	8.0b	74.0b
NFT	6.4b	63.4b
AP	19.3a	208.2a

Means with same letter are not significantly different at 5% level.

Table 4.3.2. Effect of cultivar on tuber per plant, and yield per plant

Cultivar	Tubers per plant	Yield per plant (g)
Russet Burbank	11.6 a	175.9 a
Kenebec	8.83 a	133.9 a
Atlantic	7.8 a	60.1 b
Désirée	10.9 a	166.8 a

Means with same letter are not significantly different at 5% level.

This result shows that the aeroponic system produced a significantly higher number of tubers per plant (19.3) when compare with other systems (Table 4.3.1). When mini-tubers were used as planting materials, aeroponic systems produced the highest a yield per plant (208.19g) but was no significantly difference from the bark-based system (Table 4.3.1). There was no significantly difference in the number of tubers per plant for each cultivars,

but Atlantic produced significantly lower yield per plant than other varieties (60.06g) (Table 4.3.2). From all the observations, aeroponic system is a suitable for produced high number of minituber than other systems.

4.3.4. Discussion.

The size and physiological condition of the mother tuber exerted a dominant influence on plant development, involving direct as well as indirect effects on stolon and tuber formation (Choi *et al.*, 1994; Lommen & struik, 1994; Ranalli *et al.*, 1994). Minitubers more slower to achieve canopy than normal seed tubers (Ranalli *et al.*; 1994). Slower emergence from minitubers is probably due to small amounts of tuber reserves available for growth and to slower haulm development caused by the relatively small root system of plants from the small tubers (Ranalli, 1997). Normally mini-tubers sprout one or two stems because the number of buds on each tuber is lesser than conventional tuber. The primary shoots in the minitubers appear to have apical dominance, and inhibit sprouting of the shoots from the remaining eyes (Ranalli, 1997). Lommen & Struik, (1994) and Ranalli *et al.*, (1994) have reported that minitubers produced only one primary stem after one month of growth. Unless new shoots develop it will take a long time to canopy closure. Slower canopy development allow increased the growing media temperatures, which promotes the stolon growth and branching (Ewing, 1985).

In the bark based system and the aeroponic system, yield per plant was not significantly different. This could be lower light interception by their canopy and lower potential of tuber initiation site for bark based system. The absence of mechanical resistance in aeroponic systems also enhanced stolon elongation and branching. When stolon and roots fill the container in aeroponic system, tubers are formed. Wan *et al.*, (1994) have reported same observation, tuberization does occur in solution culture when the container becomes filled with roots and stolons. Therefore the number of tubers per plant and tuber yield per plant are higher in aeroponics systems, due to the high number of tuber initiation sites. Multiplication rates per tuber are lower for minitubers, because minitubers produced fewer daughter tubers because produced lower leaf area and decreased light interception by their canopy (Lommen & Struik, 1995)

Plate 1. Potato crops in early stage in glasshouse

Plate 2. Fully developed potato crops in glasshouse

Plate 3. Potato tubers and stolon developing in the aeroponic system.

Plate 4. Potato tubers and stolons developing in the bark system.

Plate 5. Potato tubers and stolons developing in the NFT system.

Plate 6. Potato tubers and stolons developing in the deep flow system.

Plate 7. Longer and thicker stolon from hydroponics systems.

Plate 8. Infected potato plants.

Chapter five

Synchronising tuber initiation

5.1. Introduction

Understanding potato tuber initiation is important if mini-tuber production is to be improved. The time of tuber initiation in relation to other aspects of plant development is thought to play a key role in determining the number of uniform size mini-tubers. The differences in size are related to differences in the date of tuber set, and in the rate of growth. However, it is commonly believed that the rate of growth of each individual tuber fluctuates (Gray, 1973; Ahmed and Sagar, 1981; Struik *et al.*, 1988). Vreugdenhil and Struik, (1989) have reported that cessation of stolon growth is important step for tuber initiation in potato plants. As stolons in hydroponics systems did not developing encounter sufficient mechanical resistance they had extremely vigorous growth. This tends to provide more potential sites for tuber initiation because longitudinal stolon growth will continue as long as conditions are unfavourable for tuberization. Rapidly growing stolons produced tubers continuously when environmental conditions favoured tuberization.

When conditions are favourable for tuber induction, stolon growth ceases naturally, and tubers form on the stolon. The time of tuber initiation on the stolon depends on stolon characteristics and will affect the tuber size distribution. Therefore, synchronising tuberization gives more uniform tubers and could reduce the cost of production by increasing the tuber number and reducing the number of harvests in an aeroponic production system. Wan *et al.*, (1994) reported that a short-term reduction of solution pH, significantly promoted tuber initiation on potato in solution cultures, this could be a useful to investgate.

Our objective was to establish a technique for synchronised minituber initiation and production in aeroponic culture and produce uniform tubers.

5.2. Materials and methods:

The experiment was conducted from February 1998 to May 1998, at the Plant Growth Unit. Two week-old rooted cuttings were planted in an aeroponics system. All growth conditions were as described in Experiment One. Six weeks after setting up plant treatments were started. A 10 % phosphoric acid solution was used to modify the pH. Roots and stolons were immersed in different pH solutions for different time intervals in low ambient light conditions to prevent possible damage by a high light intensity. The pH and conductivity were measured and adjusted every twelve hours as necessary.

5.3. Plant materials

Aeroponically propagated plants of Russet Burbank (RB), Rua (R), and Désirée (D) were used for this experiment.

5.4. Treatments and Experimental design

A three factor factorial design was used in this experiment with two replicates.
Factor – 1 Cultivar: Russet Burbank, Rua and Désirée.
Factor – 2 pH: 3.0, 3.5, and 4.0
Factor – 3 Time: 12 hrs, 24 hrs and 48 hrs.
All treatments were randomised.

5.5. Data collection and statistical analysis

Plants were harvested 4 weeks after acid treatment, tuber number and yield per plant were recorded. Data were analysed statistically.

5.6. Results and discussion

Table-5.1 Effect of acid treatment time, pH and cultivar on tubers per plant

Time	Cultivar								
	Russet Burbank			Rua			Désirée		
	pH			pH			pH		
	3.0	**3.5**	**4.0**	**3.0**	**3.5**	**4.0**	**3.0**	**3.5**	**4.0**
12	27.0	14.5	7.0	9.5	17.0	14.0	25.0	21.5	22.5
24	6.0	23.5	16.0	34.0	50.5	12.5	38.5	19.5	20.0
48	14.5	5.5	12.5	4.0	11.5	8.0	16.0	15.0	7.5

Analysis of variance	**Probability**	**SEM**
Cultivar (df= 2)	P< 0.001	2.0
pH (df= 2)	P< 0.05	2.0
Time (df= 2)	NS	2.0
Cultivar x pH (df= 4)	NS	3.5
Cultivar x Time (df= 4)	P< 0.05	3.5
Time x pH (df= 4)	NS	3.5
Cultivar x pH x Time (df= 8	P< 0.05	6.0

df= degrees of freedom, SEM= standard error of mean
NS= non significant at P<0.05.

Table 5.2. Effect of acid treatment time, pH and cultivar on tuber yield per plant (g)

Time	Russet Burbank			Rua			Désirée		
	pH			pH			pH		
	3.0	**3.5**	**4.0**	**3.0**	**3.5**	**4.0**	**3.0**	**3.5**	**4.0**
12	194.9	283.2	121.0	134.3	225.3	115.2	306.0	346.9	456.2
24	282.5	365.6	143.2	289.6	268.3	278.0	360.5	367.7	471.1
48	104.9	30.7	105.6	132.8	279.3	177.6	270.8	159.3	201.7

Analysis of variance	**Probabilit y**	**SEM**
Cultivar (df= 2)	P< 0.05	28.5
pH (df= 2)	NS	28.5
Time (df= 2)	P<0.05	28.5
Cultivar x pH (df= 4)	NS	46.74
Cultivar x Time (df= 4)	NS	46.74
Time x pH (df= 4)	NS	46.74
Cultivar x pH x Time (df= 8)	NS	80.96

df= degrees of freedom, SEM= standard error of means
NS= non significant at P<0.05.

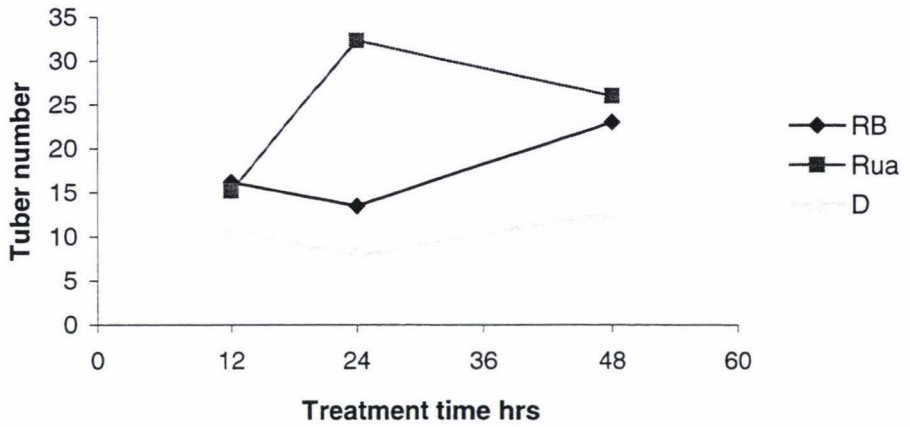


Figure- 5.1. Effect of treatment time and cultivar on tuber number

In this experiment we found that cultivar significantly affected both the tuber number and yield per plant. Cultivar Rua produced the highest yield per plant (314.0 g) and highest tuber number per plant (24.5). Cultivar Désirée produced the lowest yield per plant (162.5 g) and lowest tuber number per plant (10.5) (Table 5.1 and 5.2). The pH significantly affected the tuber number but produced no significant effect on tuber yield. pH 3.5 produced the highest tuber number per plant (19.8) and highest yield per plant (258.5 g) whereas pH 4 the lowest number of tubers (13.3). Treatment time also significantly affected the number of tubers but, produced no significant effect on tuber yield. Time and cultivar showed an interactive effect on tuber numbers (Fig. 5.1). This interactive effect was produced mainly by the cultivar, because it elicited a significant resp

RB- Russet Burbank
D- Désirée

the number of tubers produced, which was independent of both the treatr level. The pH level significantly affected the tuber number but produced, no significant effect on the tuber yield. pH 3.5 produced a higher tuber number per plant (19.83) while pH 4 produced the lowest number of tubers per plant (13.3).

Tuber initiation occurred in the plants when given a deep flow acid treatment. Tuber initiation was visible began on the 4th day after the acid treatments. The effect of the acid

treatment depended mainly on the stage of stolon development. Wan *et al.*,(1994) observed similar results in a pH 4.0 treatment, where tuber initiation was delayed and the final tuber count was significantly lower than at pH 3.5. We observed that pH 3.0 treated plants showed more stress and damage to younger stolons tips. This is a possible reason the lower number of potential sites for tuber initiation. At treatment time, we observed that the stolon maturity level differed for each cultivar.

Our preliminary experiments revealed that young plants showed more evidence of stress due to a low pH level (pH = 3) than mature plants. We observed stolon tip damage, foliar necrosis, and plants showed a wilting condition for two to three days. When there was a high temperature or more sunlight during the acid treatment time, plants became more stressed than in shaded conditions. Tibbitts and Cao (1994) reported that tuberization was inhibited when roots and stolons were immersed in solution or subjected to continuous mist culture. In contrast, this study showed following acid treatment when roots and stolons were fully developed in deep flow and aeroponic systems containers, then tubers were formed as normal. This was due to some level of mechanical resistance or stress conditions on the stolons. The stress or mechanical resistance changes the hormonal balance in the stolons causing stolon growth to cease.

Potato stolons produce ethylene under mechanical constraint in the soil. As result, stolon elongation will stop, provided that the level of gibberellins is sufficiently low (Vreugdenhil and Dijk, 1989). Lugt *et al.*, (1964) reported extremely vigorous stolon growth and a delay of tuberization when the stolon environment did not provide enough mechanical resistance for tuber initiation or only a low level of ethylene was produced in the stolons. Vreugdenhil and Struik (1989) have reported similar observations. These results indicate that a certain level of physical resistance or a stress condition is important to stop the longitudinal growth of stolons for normally-shaped tuber formation.

Further study is important to:

1. determine the optimum stage of stolon development for acid treatment.

2. determine the optimum pH level and treatment time for different stages of stolon development.
3. determine if other types of stolon stress will promote tuber induction

Chapter six

The Effect of gibbrellic acid level on sprouting of potato minitubers

6.1. Introduction.

Minitubers have a dormant period immediately after harvesting, and therefore cannot be planted immediately. They must to be stored at least until dormancy is over. Otherwise sprouting will be spasmodic). The duration of dormancy is also related directly to the date of tuber initiation, the position of the tuber on the plant during its growth, and the tuber weight and cultivar (Lommen, 1993a,b and Leclerc *et al.*; 1995).

Lommen (1993a) concluded that minitubers must be produced more than 5 months before they are planted, or dormancy has to be broken artificially. Burton (1973) showed that small, physiologically immature 'normal' tubers could suffer high weight losses immediately after harvesting. Furthermore, during storage they also show higher weight losses than larger tubers, probably because of their higher surface area to volume ratio. Lommen (1993b) showed that tubers which deteriorated during the cold-storage period had high weight losses during the curing period. There is a lack of information relating specifically to minituber dormancy and dormancy-breaking. GA could be useful for breaking the dormancy of minituber and producing multiple stems from the dormant buds. This experiment sought to identify if GA treatment helped in breaking minituber dormancy.

6.2. Materials and methods

The experiment was conducted from February 20th to April 20th 1998 at the Plant Growth Unit at Massey University.

Plant materials

Minitubers (2 – 4 g weight) of cultivars Russet Burbank (RB), Kennebec (K), Atlantic (A), and Désirée (D) were selected for this experiment. All minitubers were produced in aeroponics, deep flow, and NFT systems and stored for two months at 5°C. Tubers from each production system was combined before allocation to each traetment.

Treatments and Experimental design

Minitubers in the 2-4 g weight group were used. Fifteen tubers were selected for each treatment and four levels of GA solution were used in this experiment (0 level, 10 ppm, 30 ppm, and 100 ppm) to break the dormancy. Gibbrellic acid solutions were prepared by dissolving 100 mg of GA in 100 ml of ethanol and diluted in 900 ml water to prepare 100ppm solution. 300ml of 100ppm solution was diluted with 700ml water to get 30ppm solution. Like in manner a, 10 ppm solution was also prepared.

Selected tubers were put in small nylon net bags and soaked in GA solution for 10 minutes, then dried for 24 hours in the dark. The treated tubers were placed on a layer of pumice (2 cm) in propagation trays, and covered with pumice to the depth of two minitubers. All treatments were arranged in RB design with three replicates. Pumice was maintained in a wet condition. Sprouting was checked every two days, and the number of sprouted tubers was recorded.

6.3. Result

The number of sprouted tubers were counted up to 52 days after GA treatment and calculations were made of the percentage of tubers sprouted from the initial number of tubers. These data were analysed statistically and are summarised in the following tables.

Table 6.1. Effect of potato cultivar on sprouting of minitubers after 52 days.

Cultivars	% of sprouting
Russet Burbank (RB)	81.7 a
Kennebec (K)	52.2 b
Atlantic (A)	45.6 b
Désirée (D)	72.8 b

Means with the same letter are not differ significantly ($p<0.05$)

Table 6.2. Effect of GA levels on sprouting of minitubers after 52 days.

Gibbrellic acid level	% of sprouting
0 ppm	32.8 c
10 ppm	65.0 b
30 ppm	74.4 ab
100 ppm	80.0 a

Means with the same letter are not differ significantly ($p<0.05$)

Table 6.3. Effect of GA levels on number of days to 50% sprouting

Gibbrellic acid level	50% sprouting
0 ppm	37.1 a
10 ppm	19.4 b
30 ppm	19.7 b
100 ppm	20.8 b

Means with same letter are not differ significantly ($p<0.05$).

Table 6.4. Effect of variety on number of days to 50% sprouting.

Cultivar	50% sprouting
Russet Burbank	19.75 b
Kennebec	24.00 b
Atlantic	32.50 a
Désirée	20.75 b

Means with the same letter are not differ significantly ($p<0.05$)

The results from this study showed that GA treatment significantly sped up the sprouting of minituber compared to the control. Fifty percent of the GA treated-tubers sprouted within 20 days after treatment, while untreated tubers took 37days.The length of time required to achieve fifty percent tuber sprouting was not significantly different at any level of GA treatment, but was significantly different from the control (Table 6.3). Overall, sprouting was highest for 100 ppm treatment, but was not significantly different 30ppm (Table 6.2). Russet Burbank produced a significantly higher sprouting percentage (81.7%) than did the other cultivars. Atlantic showed poorer sprouting than did the other cultivars (Table 6.1). The highest and lowest sprouting cultivars were Russet Burbank and Atlantic respectively. These results indicate that GA treatments positively induce tuber sprouting.

When sprouting data were combined for each cultivar and plotted against log [GA] then an almost linear function was revealed. As GA concentration increased for 0 –10 ppm sprouting increased by ≅ 30%, and between 10 – 100ppm by ≅ 15% concentrations increased by log 0.5ppm sprouting percentage also increased by about 10% (Fig. 6.1)

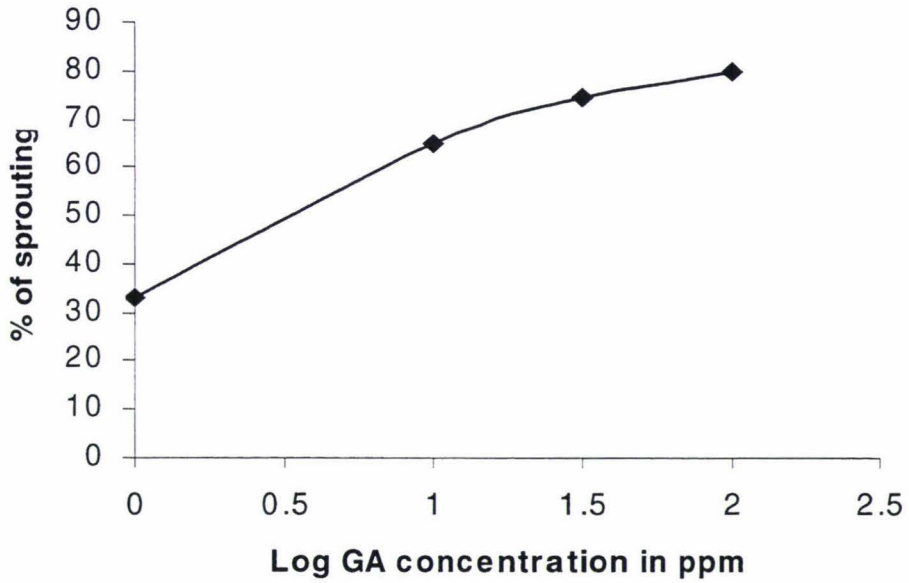


Fig 6.1. Effect of GA concentration on sprouting of minitubers.

6.4. Discussion

Minitubers show a dormant period immediately after they are harvested, and therefore cannot be planted immediately (Lommen, 1993a,b). Gibberellic acid promotes growth and branching of stolons on potato crops (Bodlaender and van de Waart, 1989) and it could act in the same way on minituber sprouting.

Lommen (1993a) has reported that minitubers took 5 months for 50% sprouting of the tubers, that it was possible to shorten the dormancy of minitubers by cold storage. Cold storage periods reduced sprouting by only 1-2 weeks (Lommen (1993a,b). Lommen and Struik (1993) suggested that minituber performance may be poor if they are produced 6 or 7 months or less before planting. Our experimental results showed that tubers given two months cold storage and GA treatment took only 20 days for 50% sprouting. Therefore, GA treatment is one method, which can be used successfully to break the minituber dormancy, and possibly enhance the production of multiple shoots.

Additional studies are needed in the following areas be fore the further use of the GA treatment can be recommended.

1. To evaluate the field performance of GA treated tubers.
2. To investigate the effect of GA treatment on tubers of different stages of physiological development.

Chapter seven

The effect of IBA level and application time on the propagation of potato cuttings an aeroponic system.

7.1. Introduction

Using stem cuttings as a source of good quality planting material requires simple, low- cost establishment methods. Stem cuttings from potato plants can be rooted in seven to fourteen days in a bark medium (Personal observation). Using the aeroponic system for plant propagation also has potential for using stem cuttings. An aeroponic system incorporates extra aeration and nutrient supply for root development and growth. Ample amount of oxygen and water are a vital to root development. However, hydroponically (but not aeroponically) produced plants develop "water roots" which suffer from transplant shock and cause a setback from normal growth. Aeroponic systems can probably provide more oxygen and water for stem base than can other hydroponics systems (Deep flow and NFT). Aeroponic technology for plant propagation and growing is now a commercial reality for the greenhouse grower. Aeroponic systems can help increase rooting of cuttings with a high survival rate, and produce more plants in less time and space, with negligible water loss. Aeroponically-propagated seedlings or cuttings can be transplanted in different systems without any stress or root loss (Personal observation).

7.2. Materials and methods

The experiments were conducted from October to November 1997 in the Plant Growth Unit at Massey University. Three galvanised containers (dimensions 120 cm x 75 cm x 75 cm) were used to set up the aeroponics system for plant propagation. Inside the container was lined with double-sided (black and white) polythene sheet (Panda film). The top of the container was covered with a wooden frame to hold cell trays. The bottoms of all cells were modified to hold the cuttings while rooting took place, to enable the cuttings to be

pushed through into the aeroponic chamber. Nozzles were fixed 15 cm apart around the top of the container. Water was sprayed continuously and recycled. These systems set-up under 50% shade cloth with a mist system. Two nozzles were fixed on each side of the propagation tent, and set for misting every five minutes for 20 seconds.

A 100-litre water tank was placed in the ground with the top of the tank lower than the drainage line. The tank was covered to exclude light and reduce contamination. Water was fed continuously to the supply line by a water pump (Mono, model CP25). The water then flowed by gravity down the drainage line and back to the tank.

Plant materials

Cuttings were obtained from cultivars of Russet Burbank (RB), Rua ® Kennebec (K), Atlantic (A), and Désirée (D). Apical shoots with 3-4 inter nodes of cuttings were used for this experiment. Cuttings were harvested from 12-weeks-old plants growing in bark, aeroponic, NFT and deep flow hydroponics systems. Cuttings from the different sources were combined prior to propagation.

Treatment and Experimental design:

An RCB design was used to examine the effect of the IBA levels and treatment times on aeroponically-propagated potato cuttings. There were 25 treatment combinations, with two replicates. Ten cuttings were used for each treatment. Treatments were
5 cultivars, two levels of IBA (0.1 and 0.3%) and two auxin treatment time intervals (1hr and 24hrs) compared with controls. Cutting was assessed after 5 days form the treatment.

7.3. Result:

The effect of the IBA treatments on rooting of potato cuttings.

Table 7. 1. Effect of IBA treatments on % root length distribution

Treatment	Roots >20mm	Roots <20mm	No roots
0.1% IBA for 1 hr	78 a	19 a	3 b
0.1% IBA for 24 hrs	78 a	18 a	4 b
0.3% IBA for 1 hr	83 a	11a	6 b
0.3% IBA for 24 hrs	79 a	18 a	3 b
No IBA (control)	53 b	18 a	29 a

Means with the same letter are not differ significantly ($p<0.05$).

Table 7. 2. Effect of cultivar on % root length distribution.

Cultivars	Roots >20mm	Roots <20mm	No roots
Russet Burbank	76 b	21 a	3 b
Rua	63b	27a	10 b
Kenebec	76 b	18 a	6 b
Atlantic	60 b	16 ab	24 a
Désirée	96 a	2 b	2 b

Means with the same letter are not differ significantly ($p<0.05$).

Plate 9. Potato cuttings in aeroponic system under mist

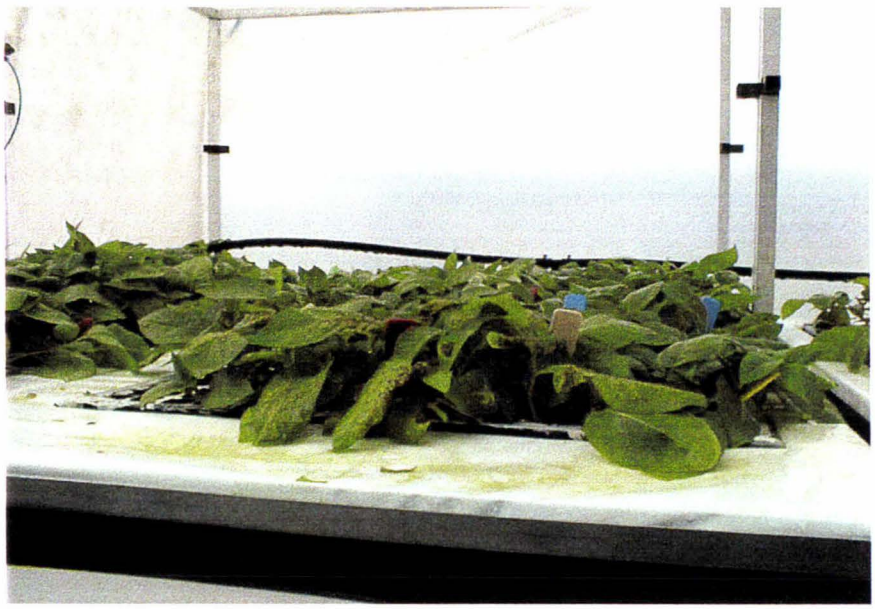


Plate- 10. Aeroponically propagated potato cuttings.

Plate- 11. Rooted cuttings from different treatment.

The overall average of rooted cuttings was 95%, using IBA treatment of 0.1to 0.3 % for 1 and 24 hours but there were no statistical differences as between the IBA level and treatment time period. A significantly poor rooting pattern was observed without IBA treatment. The cultivar Désirée produced a significantly higher percentage (96%) of long roots than the did other varieties, while cultivar Atlantic showed a poor rooting pattern when compared with the other cultivars.

7.4 . Discussion

The use of auxins to promote adventitious root formation on cuttings is routine in many production nurseries. IBA is a rooting hormone, which induces root formation and speeds root growth and elongation. It is an easily applied treatment, and is effective for many plant species. Normally, some IBA treatment is probably better than none (Carter and Slee, 1993). Our results indicate that about 79 –83 % of IBA-treated cuttings rooted and produced longer roots than those under the control treatment. This observation indicates that an IBA treatment is important even in an aeroponic propagation system, and beneficial for easily rooting plants such as potato. Vander Zaag *et al.,* (1990) have reported that

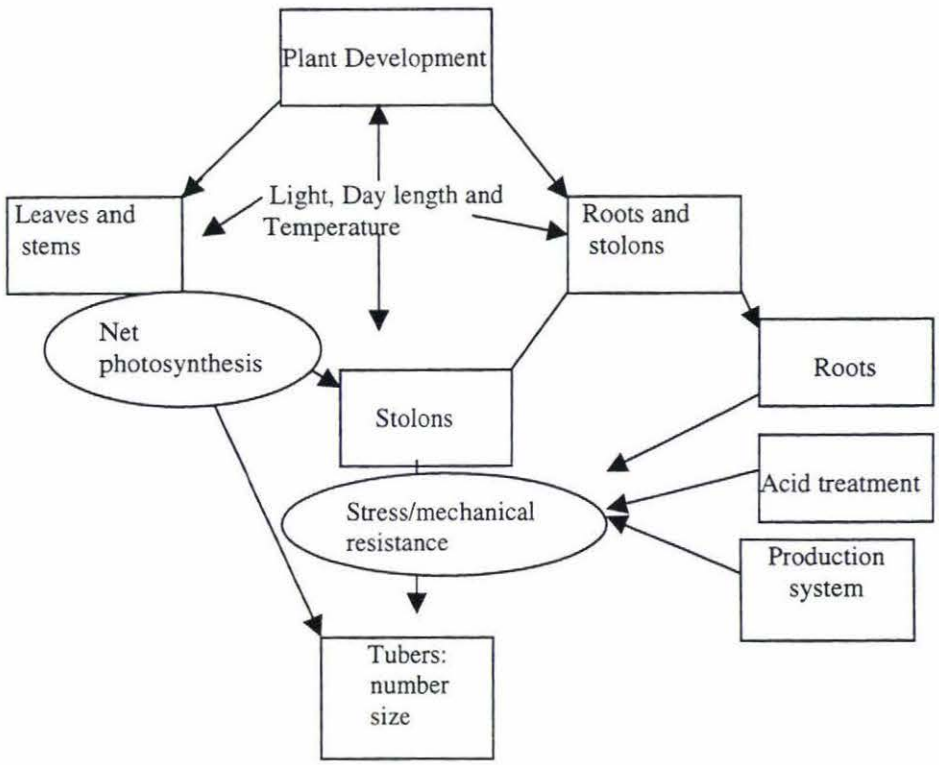
potato cuttings can be rooted and established without the aid of hormones, but he did not mention the time for rooting without the aid of hormones. We observed that IBA treated cuttings rooted earlier than did the controls. The treatment time did not show any significant effect on root formation. Cuttings in the aeroponics propagation system all appeared as turgid, suggesting that water loss and water uptake in the cuttings were in equilibrium throughout the study.

When the same experiments were repeated four weeks after the first experiments, a stem rot developed at the basal end of the cuttings. The first experiments used solid, shorter inter-node and hardy cuttings, compared to the second experiment-which used hollow, long and succulent cuttings. The environmental conditions were also different from those in the first experiments, (longer photoperiods and higher temperature) due to a different time of the year. High temperature and moisture seem to provide an ideal environment for *Pythium spp.* frowth (Favrin *et al.*, 1988). From these experiments, it was observed that robust and short cuttings are most suitable for aeroponic propagation systems, and that IBA treatment also promotes the rooting of cuttings.

Chapter eight

General conclusion

Model 8.1. Simple model for tuber production

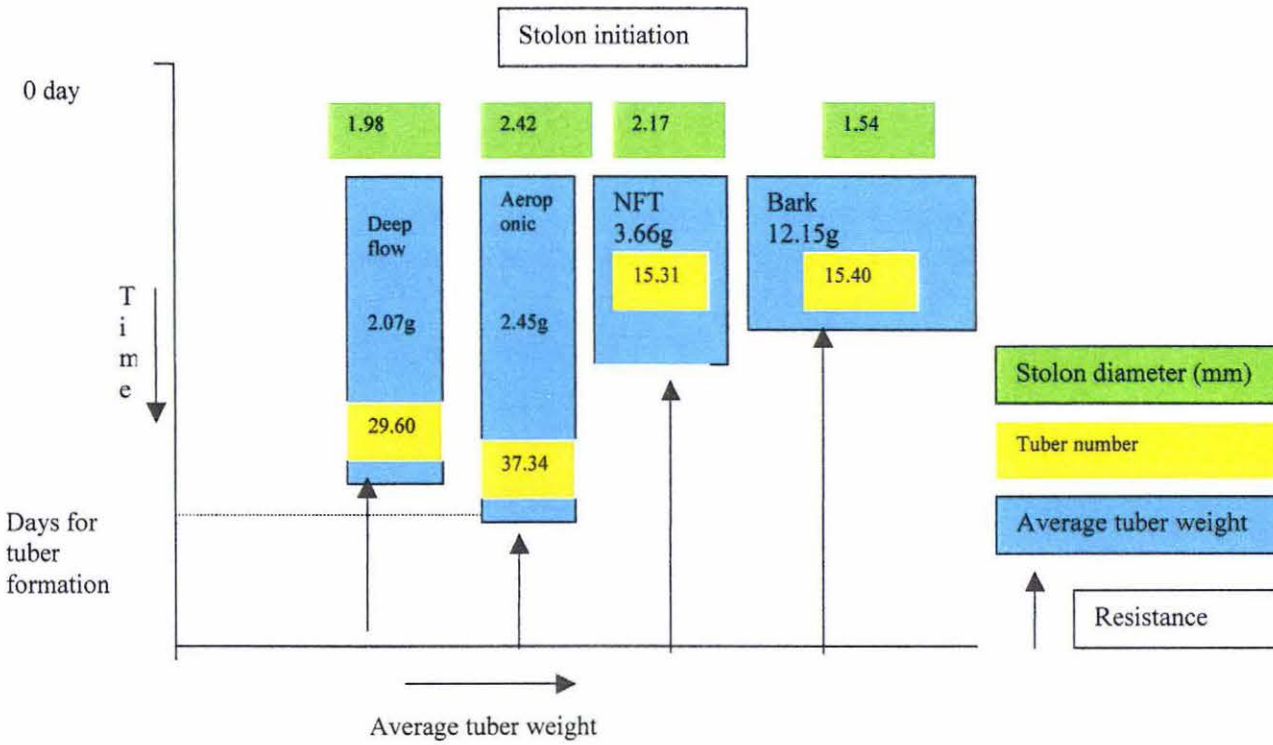


This simple model can explain all the processes of tuberization and how all components interact in this process. While there are many components to this model our studies involved an examination of different production systems and their effect on the tuber numbers and tuber yield per plant. We found that aeroponic and deep flow systems produced more stolons, and delayed tuberization. When the acid treatment was used, tuber initiation was visible 4 days after treatment, and a high number of uniform size tubers were achieved by harvesting the time.

The tuberization pattern varies in different systems as well as for different planting materials. In this model, plant development is influenced by the type of planting materials,

production systems and environmental conditions. We observed that when *in vitro* plants and minitubers were used as planting materials, plant development was slow at the early stage-but, when rooted cuttings were used as planting materials the plant development was high this is stored reserves in each propagule. In our studies although we did not set out to compare the planting materials in different production systems, to obtain enough experimental material we used different planting materials in our experiments at different times.

Model 8.2. The following models explain how each system influence on tuber production.



This model explains different systems how influence the average tuber weight, tuber per plant and stolon diameter. We observed stolon initiation time for all systems was the same but, the tuber initiation time differed for each system. Bark and NFT systems formed tubers earlier than deep flow and aeroponics systems because bark and NFT systems produced higher average tuber weight. Tuber initiation in potatoes occurs readily in solid matrix culture (Tibbitts and Cao, 1994; Tibbitts and Wheeler, 1987) and efficiently with NFT (Wheeler *et al.*, 1990). In aeroponic and deep flow systems, stolons were freely hanging in buckets, but in NFT systems, stolons grew on the NFT channel surface. NFT channel surfaces may provide some resistance for the stolons which stimulates the tuber initiation.

The length of time prior to tuberization determines the number of tubers per plant. When tuberization was delayed the increased number of tubers per plant increased. In aeroponic and deep flow systems, the length of time prior to tuberization was high, which produced

more tubers than did bark and NFT systems. When tuberization was delayed, stolons grew continuously in deep flow and aeroponic systems, and produced more tuberization sites. We found that, when stolons and roots filled the growing container tubers formed in aeroponics and deep flow systems. It is possible that, if we had used larger containers, then we might have delayed onset of tuberization and continued stolon development until the stolons and roots filled the container. This possibility needs to be studied further to develop the most suitable aeroponic system for minituber production.

Stolons grew continuously in hydroponics systems, because there was no mechanical resistance to stop longitudinal stolon growth. The degree of mechanical resistance encountered by the extending stolon may affect the stolon and tuber development (Vreugdenhil and Struik, 1989). In the hydroponics systems, because developing stolons failed to encounter sufficient mechanical resistance, they had extremely vigorous growth. This tends to provide more potential sites for tubers. Longitudinal growth of a stolon will continue as long as condition are unfavourable for tuberization. The rapid growing stolon produced tubers continuously when environmental conditions favoured tuberization. Long day (LD) conditions favour stolon elongation, whereas short days (SD) result in cessation of stolon growth (Chapman 1958).

When considering photosynthesis partitioning, it may vary in different system. This reflects on tuber average weight and stolon diameter. When stolon diameter increase, average tuber weight decreased in this model. In hydroponics system, part of the photosynthtate is used for stolon development and growth. The arrow mark in (Model- 8.2) the mechanical resistance in the growing systems. The bark system provides more resistance to the stolon whereas the aeroponics system provides less resistance. In this model, if the system provided more resistance to the stolon, then the plant reduced the time prior to tuberization. Again it reflects on the number of tubers per plant.

When the tuber size distribution was considered, the hydroponics systems produced a higher percentage of 1-2 g size tubers than did the bark system. These results clearly indicate that tubers are formed continuously in hydroponics systems which may be related

to continuous development of stolons in hydroponics systems. Gray (1973) stated that a removal of the mechanical resistance in an early stage of plant development induced the formation of secondary stolons and numerous small tubers. This statement supports the tuberization pattern in aeroponic and deep flow systems. Within the hydroponics systems the NFT system produced a lower percentage of 1-2 g size tubers (51.9%) at the time of harvesting.

In these studies we found that aeroponic system was suitable for minituber production, as numbers of tubers per plant were high. The aeroponic system produced a high percentage (82%) of 1- 2g group tubers. This size is very convenient for storage and transport. Yield per plant, average tuber weight, and largest tuber weight were higher in bark systems possibly because in bark systems there is enough mechanical resistance to control stolon growth and supply the net photosynthesis to tuber growth when compared the hydroponics systems. The reduced mechanical resistance in hydroponics systems produced thicker (1.92-2.42 mm) and longer stolons than did bark systems.

Mini-tubers and rooted cuttings can be used for mini-tuber production in hydroponics systems, but the yield potential is low, because the number of stolons per plant was lower for plants from minitubers and rooted cuttings. The number of stolons from rooted cuttings was dependent on the number of nodes in the medium or system. *In vitro* potato plants usually produced a greater number of stolons in the aeroponic system.

We used acid treatment to synchronise the tuberization in aeroponic systems, because in these, stolons grew continuously and tuber formation was delayed. We found that, four days after acid treatment, the tuber had formed on the stolon. This process depends on the cultivar specific or stage of stolon development. From the above model, we can modify length of time to tuberization by acid treatment or any kind of stress treatment to increase the ethylene level in the stolon. Ethylene production in plant tissues increases with various types of stress. Potato stolons produced ethylene upon mechanical constraint in the soil (Vreugdenhil and Struik, 1989). Acid treatment could increase the stress on the stolon and produce ethylene, as a result, stolon elongation ceased and tuber formation was initiated in the aeroponic system.

Further studies are important for determining the effect of acid treatment on the stage of stolon development and pH level. It will determine the number of tuberization sites or the number of tubers per plant, and the average tuber weight.

Lommen (1993a) has reported that mini-tubers took 5 months for 50% sprouting of the tubers, and it was possible to shorten the dormancy of minitubers by cold storage by only 1-2 weeks. Our experiments' results showed that two months' cold storage and GA treated tubers took only 52 days for 80% sprouting. Therefore, Gibberellic acid treatment is one method, which may be used to break the minituber dormancy and possibly enhance multiple shoots. Minitubers sprout one or two stems, because there are fewer buds on the tuber. Application of GA or other plant growth-regulators treatments could perhaps, increase minituber sprouting. The way in which storage time and GA treatment affect tuber dormancy are topics that require further study.

Application of IBA to potato cuttings induced root formation and root growth. About 79 – 83 % of IBA treated cuttings rooted and produced longer roots than untreated cuttings. Therefore, rooting hormone treatment is important for aeroponic propagation systems to reduce the rooting time. When cuttings were in the aeroponics propagation system, all cuttings appeared to be as fully turgid which meant that water uptake and water loss were in equilibrium under the aeroponic system. Hollow and succulent cuttings are not suitable for aeroponic propagation systems, because most of the cuttings got stem rot.

The aeroponics system is a suitable system for minituber production, because the number of tubers and the percentage of 1-2g sized tuber were higher than in other systems. A high level of sanitation and of control of the solution temperature during the day time is important in hydroponics systems, because these factors control plant-growth and diseases. Continuous harvesting is possible in aeroponic system with minimal plant disturbance. Aeroponics systems could be used for rooting stem cuttings.

The aeroponic system could be incorporated as a part of the current seed potato production system to efficiently generate a large number of prebasic tubers.

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