CHAPTER 5

CUTTINGS AS A MODEL OF PLANT REACTION TO INDUCTIVE CONDITIONS

5.1 Introduction

Very little is known about the control of tuberisation in *Plectranthus esculentus*. Tuber induction in many species is under photoperiodic control, with the stimulus being perceived by the leaves (Gregory, 1965; Menzel, 1985). The response to photoperiod is affected by environmental factors such as temperature, light intensity, and level of nitrogen fertilisation (Werner, 1935; Went, 1959; Bodlaender, 1963; Gregory, 1965; Marinus & Bodlaender, 1975; Kraus, 1978; Bodlaender, 1981; van der Zaag & van Loon, 1987; Vreugdenhil & Struik, 1989; Ewing & Struik, 1992), as well as genotype (Ewing & Wareing, 1978; Mendoza & Haynes, 1977; Steer, 1980). A problem encountered when investigating the effects of environmental conditions on tuber induction is that the size of the plant can influence the results (Ewing, 1981; Kahn, Ewing & Senesac, 1983).

Although potato tubers normally form on underground stolons, it has been found that any shoot apex, including apical and axillary buds, floral buds, stolon tips, and sprouts on tubers is capable of tuberising (Knight 1806, 1809; de Vries, 1878; Vöchting, 1887; Wellensiek, 1929; Werner, 1954; Ewing, 1985). Observations of *Plectranthus esculentus* plants, both in the field and under controlled conditions, has revealed that axillary buds in the leaf axils of the stem, as well as the terminal bud at the shoot apex are capable of forming aerial tubers (Figure 3.2a, b). This reaction is similar to that which has been noted in potato (Cox, 1967; Gregory, 1956, Ewing, 1985; Ewing & Struik, 1992).

Although the tuberisation ability of cuttings has long been known (Vöchting, 1887; Kupfer, 1907; Isbell, 1931), it was Gregory (1956) and Chapman (1958) who demonstrated the true potential of this technique (Ewing, 1985). Gregory (1956) showed that the presence of the tuberisation stimulus in potato could be demonstrated by making cuttings from the stem.

The cuttings reflected the changes brought about in the entire plant by the induction stimulus, so providing a simplified model of tuberisation. These results were confirmed by Chapman (1958), who also conducted experiments on the graft transmission of the induction stimulus, as well as showing that the movement of the stimulus was in a basipetal direction.

The technique was expanded by other researchers working on various aspects of tuberisation in potato, including the possible role of the mother tuber in tuberisation (Madec & Perennec, 1962). A great deal of the investigations into *Solanum tuberosum* ssp. *andigena* has also been carried out using this technique (Kumar & Wareing, 1972,1973; Woolley & Wareing, 1972a,b). The technique has since been used with success by other researchers investigating potato tuberisation (Ewing & Wareing, 1978; Ewing, 1985; Ewing & Struik, 1992). A great advantage in using cuttings rather that entire plants is that the effect of plant size on the results is negated (Ewing, 1976; Ewing, 1978; Ewing & Wareing, 1978; Ewing,1981; Kahn & Ewing, 1983; Kahn *et al.*, 1983).

As all axillary buds on the stem of *Plectranthus esculentus* have the potential to develop into tubers, the use of cuttings may be a suitable alternative to the use of entire plants for tuberisation studies. The objectives of this study were to establish whether;

- stem cuttings act as reliable models of tuberisation in *Plectranthus esculentus*
- size and/or position of cutting on the stem affects its reaction
- cuttings from non-induced plants could be induced to tuberise.

5.2 Materials and Methods

Pilot studies indicated that *Plectranthus esculentus* did not initiate tubers when exposed to a 14 hour photoperiod, but readily formed tubers under 10 hour photoperiods. These two extremes of photoperiod were used to investigate tuberisation on cuttings. Two experiments were carried out to investigate the use of cuttings.

Experiment 1 - The use of cuttings as indicator of tuber induction

Intact plants were exposed to inductive (10 hour photoperiod) or non-inductive (14 hour photoperiod) conditions for a period of 14 days, after which cuttings were taken. Both cuttings and intact plants were then kept under non-inductive conditions for a 21 day period to allow tuber growth to take place. The experiment was laid out as a fully randomised design with eight replicates.

Experiment 2 - A comparison of the reactions of cuttings and intact plants

In this case the cuttings were taken from non-induced plants and subsequently exposed to inductive or non-inductive conditions. Intact plants were simultaneously exposed to these conditions. After a 14 day treatment period the plants and cuttings were kept under non-inductive conditions for 21 days for tuber growth to take place. The experiment was laid out as a fully randomised design with six replicates.

The trials were carried out at the phytotron facility on the experimental farm of the University of Pretoria. In order to negate the effect of genotype on the results all material used originated from a single mother plant. Multiplication took place *in vitro* using the procedure as described in **Chapter 4**. After hardening off, plants were allowed to grow for a period of eight weeks in an air-conditioned glasshouse set to a 25/20°C day/night temperature regime and a daylength of 15 hours. After this preceding growth period the plants were transferred to the plant growth cabinets for the photoperiodic treatments.

All trials utilised 1 litre black polyethylene pots 11cm high with a 10cm diameter. The base of each pot was lined with brown paper prior to filling to prevent sand running out the drainage holes. Standard quartz sand as used in sand filters, with an average particle size of approximately 2mm, was used. Sufficient water to ensure free drainage of water was applied to the pots prior to planting. Planting took place once no more water drained from the base of the pots. After planting of the cuttings the pots were placed in a 25 x 40cm polythene bag. Two 45cm lengths of galvanised steel wire, bent into a U-shape, were inserted into the pots and a polythene bag sealed over this frame by knotting to form a mist chamber. Bags

were opened every 4 days in order to ensure sufficient aeration.

Two Conviron Controlled Environment type PGW36 growth cabinets as described by Hammes, Beyers & Nel (1973) and Beyers, Hammes & Nel (1976) were used in these experiments. One was set to induce tuber formation by exposing the plants to short days with a 10 hour photoperiod, while the second cabinet was set to supply 14 hours of continuous light (non-inducing conditions). Pilot studies indicated that *Plectranthus esculentus* did not initiate tubers when exposed to a 14 hour photoperiod, but readily formed tubers under 10 hour photoperiods. A 25°/17°C day/night temperature regime on a 12/12 hour cycle was maintained.

After completion of the treatment period the pots were transfered to an air-conditioned glasshouse set to a 25/20°C day/night temperature regime. Supplementary lighting was provided to illuminate the glasshouse from 06:00 to 08:00 and again from 16:30 to 21:00. This ensured that plants were exposed to 15 hours of light, a period shown by pilot trials to be more than sufficient to prevent tuber initiation in this species. A SON 400W lamp was suspended from a steel beam below the glass roof at a height of approximately 2.6m from the floor at the front of the glasshouse. This was supplemented by four 150W incandescent bulbs together with three 1.2m fluorescent light fittings, each containing two 40W cool white tubes. These were fixed 2.7m above the floor on the opposite side of the glasshouse. All lights were operated by timers.

Four different types of cuttings were used, with two being planted in two different orientations, giving a total of six treatments for each inductive condition. Together with the intact plants this provided a total of 14 treatment combinations. Cuttings were prepared in the following way:

Apical cuttings

These consisted of the stem apex and three fully unfolded leaf pairs. The pair of leaves from the lowest node were removed at the natural abscission point, and the base of the cutting inserted into the moist sand to a depth midway between the two lowest nodes.

Two-node sub-apical cuttings

These consisted of the first two nodes below the apical cutting. The leaf pair from the lowest node were removed and the base of the cutting inserted into the moist sand to a depth midway between the nodes.

Single node cuttings (vertical)

The node following the two-node sub-apical cutting with both leaves intact planted vertically with 50% of the leaf lamina extending above the surface of the sand.

Single node cuttings (horizontal)

The next node, with one leaf removed, planted horizontally with 50% of the leaf surface extending above the surface of the sand.

Half-node cuttings

The node was separated down the middle of the stem to form two half-node cuttings. One of these was planted vertically, and the other horizontally, both with 50% of the leaf surface extending above the surface of the sand.

On completion of the growth period cuttings were harvested and the following data collected:

occurrence of subterranean tubers,
tuber growth from aerial axilliary nodes,
number of aerial tubers,
length of aerial tubers,
mass of aerial tubers (fresh and dry),
number of subterranean tubers,
length of subterranean tubers,
mass of subterranean tubers (fresh and dry),
occurrence of tuber buds, and
occurrence of swollen bases on shoots (both aerial and subterranean).

All data were submitted to the ARC- Agrimetrics Institute for statistical analyses using the Genstat Statistical Analysis program.

5.3 Results and discussion

Experiment 1 - Use of cuttings as indicator of tuber induction

In this experiment the entire plants were exposed to inductive and non-inductive conditions for a period of 14 days, after which cuttings were made and allowed to grow for a period of 21 days. Tuberisation manifested as swollen bases on underground shoots (Figure 5.1a). Tuber buds started to develop on these swollen bases (Figure 5.1b), and increases in size of these buds resulted in clearly identifiable tubers (Figure 5.1c). The percentage cuttings exhibiting swollen bases on underground shoots and the number of developing tubers were recorded. No indication of tuber formation was noted on any of the treatments that had been exposed to non-inductive conditions. Figure 5.2 shows the tuberisation reactions to inductive conditions for the various types of cuttings.

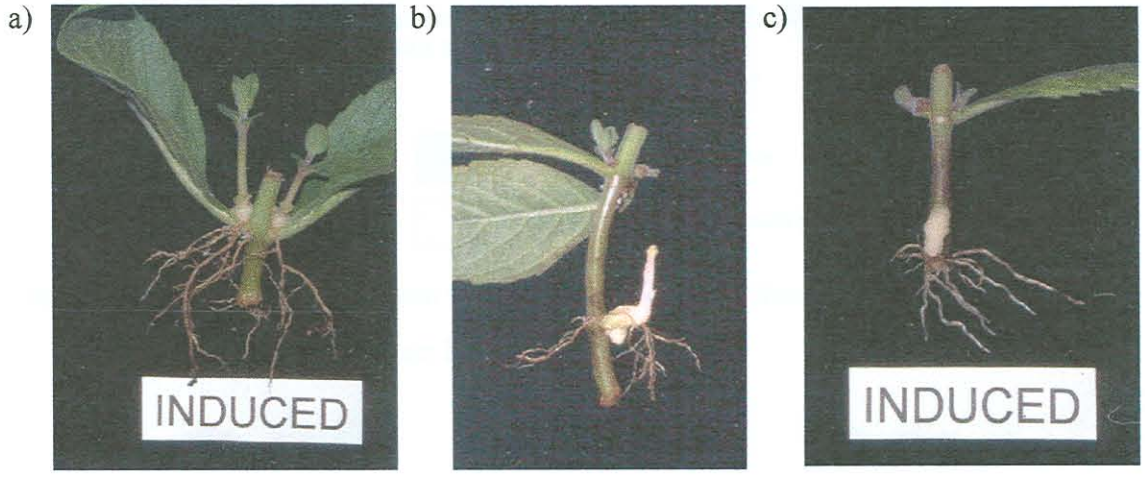

Figure 5.1 Examples of swollen bases on underground shoots (a), a tuber bud on the swollen shoot base (b), and a tuber (c)

Swollen bases on underground shoots

Apical cuttings reacted poorly to inductive conditions, producing no tubers and with only 12% of the cuttings showing swollen bases on the underground shoots. Only 50% of the apical cuttings from non-induced plants developed underground shoots (data not shown). Cuttings from plants exposed to non-inductive conditions exhibited no signs of tuber induction. The low number of shoots produced on the apical cuttings could be due to apical

dominance.

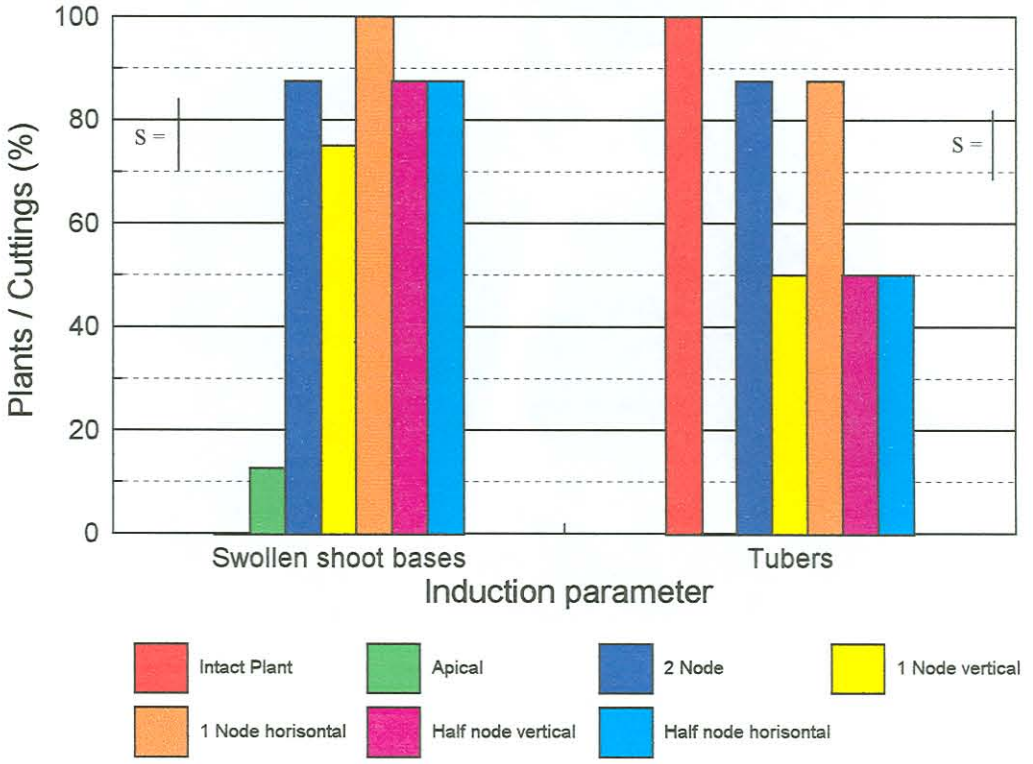

Figure 5.2 Tuber induction on various types of cuttings from induced plants compared with that on intact induced plants

All intact plants exposed to inductive conditions produced tubers. Some swollen bases were observed on the lower aerial branches (Figure 5.3).

The two-node, and both types of half-node cuttings produced underground shoots, and on 88% of these cuttings the bases of the shoots were swollen. All of the single node cuttings produced underground shoots, and 100% of those on the vertically planted cuttings exhibited swollen bases, while 75% of the horizontally planted single node cuttings exhibited this sign of tuber induction.

Figure 5.3 Swollen shoot base of an aerial shoot on an intact plant exposed to inductive conditions

Tubers

No tuber development was noted on intact plants or cuttings from plants exposed to non-inductive conditions. Tubers developed at the base of the stem of all intact plants exposed to inductive conditions (Figure 5.2). None of the apical cuttings from induced plants showed any sign of tuber development, while all of the other types of cuttings showed at least some tuber development. On the two-node and single-node horizontally planted cuttings 88% of the cuttings developed tubers. Tubers formed on 50% of each of the other types of cutting (single node vertical, and both orientations of half-node cuttings).

All cuttings, with the exception of apical cuttings, showed similar reactions to inductive conditions as the entire plant. Although any of these cutting types could be used in experiments to observe the effect of inductive conditions on *Plectranthus esculentus* plants,

the best results were obtained from the two-node and horizontally planted single node cuttings. However, from a practical point of view it is easier to work with two-node cuttings.

Experiment 2 - A comparison of the reactions of cuttings and intact plants

In this experiment cuttings from non-induced plants were exposed to inductive or non-inductive conditions. Intact plants were exposed to the same conditions as the cuttings. The length of time between taking the cuttings and harvesting resulted in desiccation of the half-node cuttings. Low survival rates (20%) resulted in the data from single-node horizontally planted cuttings being discarded from the final analyses. Consequently, results are only presented for the apical, two-node and single node cuttings.

Tuberisation

No signs of tuber formation were noted on any of the non-induced treatments. From Figure 5.4 it can be seen that all the intact plants formed tubers, while none of the various cuttings tested showed this magnitude of reaction to the inductive conditions. Apical cuttings had no tubers whatsoever, while only 40 % of the two-node and single-node vertically planted cuttings produced tubers. When these results are compared with those of the previous experiment it can be seen that cuttings from non-induced plants exposed to inductive conditions did not tuberise to the same extent as those from intact plants exposed to the same conditions.

The differences in tuber formation may be explained by the leaf area of the various treatments. In potato it has been found that leaf area plays a definite role in tuberisation, and the larger the plant the greater the tuberisation reaction, particularly under marginal conditions (Ewing, 1985). It has been postulated by a number of researchers that a larger leaf area would result in much more of the "tuberisation stimulus" being transported to the base of the plant, and such plants would then show signs of tuberisation before smaller plants, even if the latter had been exposed to more favourable inductive conditions (Kahn

et al., 1983). A larger leaf area should also result in the availability of more assimilates for tuber growth, resulting in faster tuber growth. The same scenario appears to be valid in *Plectranthus esculentus*, as indicated by these results.

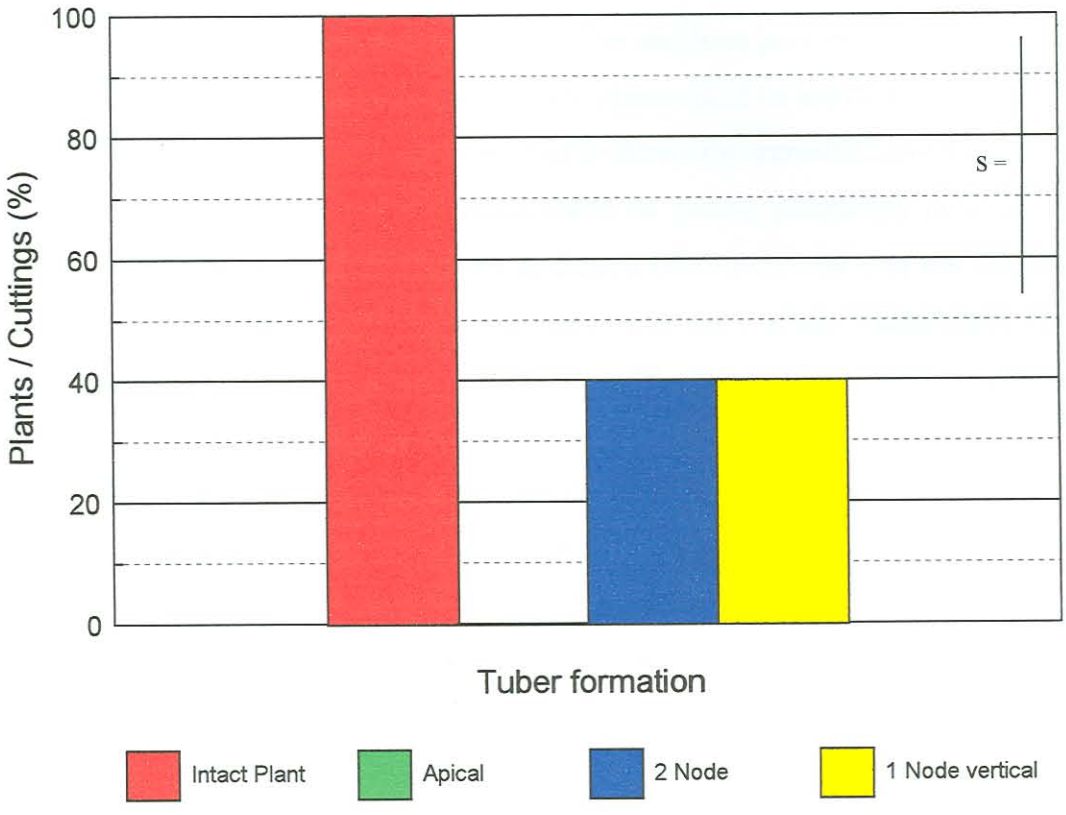

Figure 5.4 Tuber formation exhibited on various types of cuttings and intact plants exposed to inductive conditions

Although the apical cuttings in both experiments had greater leaf numbers and areas than the other cuttings, the leaves were not fully developed and were smaller than those on the two-node and single-node cuttings. It is possible that the lack of fully developed leaves could have affected the tuberisation on the apical cuttings. The young leaves are major sites of active gibberellin synthesis, and their activity is very high in the growth points of plants where active growth is taking place (Salisbury & Ross, 1978). In potato it has been postulated that the tuberisation process is controlled by a balance between gibberellins and

other endogenous growth substances, and that tubers are initiated as soon as the gibberellin content decreases below a certain threshold level (Hammes, 1971; Hammes & Nel, 1975). This would explain why the presence of fully developed leaves would enhance tuberisation in potato cuttings, as found by Kahn *et al.* (1983). It is possible that similar processes to those in the potato play a role in *Plectranthus* tuberisation, as an excessive gibberellin level produced by the young leaves and apical meristem may have prevented the apical cuttings tuberising. Hammes (1971) showed that potato plants could be induced to tuberise under non-inductive conditions by the application of 2-chloroethyltrimethylammonium chloride, a gibberellin inhibitor, but that the effect could be almost completely nullified by the application of gibberellic acid. Hammes & Beyers (1973) showed that the response of potatoes could be manipulated by the removal of the growing tip and young leaves, but that this response could be reversed using gibberellic acid applications.

It is possible that the 14 day exposure to inductive conditions was not sufficient to reduce the gibberellin level in the meristematic tissue in the youngest part of the plant below the threshold value required for tuberisation to take place, while sufficiently low levels of gibberellins were found in older portions of the plant to allow tuberisation. The effect of plant hormones on the tuberisation process in this species needs to be examined in order to check this hypothesis.

All of the induced intact plants with their large leaf areas produced tubers, while a lower percentage of the cuttings were able to produce tubers under the same conditions in the second experiment. Hammes & Beyers (1973) found that exposing the entire plant, rather than portions of the plant, to photoperiod treatments resulted in a better tuberisation effect in potato, showing that tuberisation is controlled by both young and old leaves. This would explain the poor results obtained when cuttings were exposed to inductive conditions. A further effect could have been the production of gibberellins in the young shoots growing from the axillary buds in the leaf axils. Differences in tuberisation found between intact plants and cuttings could also be partially explained by the differences in leaf area. In potato it has been postulated that the low leaf area on cuttings would result in a low production of the required "tuberisation stimulus" during short periods of exposure to inductive conditions

(Ewing, 1985). The smaller leaf area of the cuttings results in smaller amounts of assimilates being made available for tuber growth, and leads to the conclusion that longer growth periods after exposure to inductive conditions would be required in order to produce good sized tubers.

The second experiment showed that longer exposure periods could be required to obtain good results with tuber growth due to the small leaf areas on the cuttings. It is therefore not possible to use this procedure to approximate plant reaction to inductive conditions.

It is clear from the results of these experiments that tuberisation is a very complex phenomenon, and requires a great deal more work to explain it in this species. It could be that similar results regarding leaf area and/or leaf ages as those obtained in potato are obtained with cuttings from *Plectranthus*, but this needs to be investigated in more detail. The localisation of the site of perception of the tuberisation response should also be determined, together with the role played by various growth substances.

Comparing the results of the two experiments it is clear that exposing the entire plant to inductive conditions prior to taking the cuttings is the approach that should be followed in future experiments using cuttings to investigate tuberisation in *Plectranthus*. Manipulation of such cuttings will hopefully shed more light on tuber formation in this species.

5.4 Conclusions

The following conclusions can be reached from the results of these experiments:

- Cuttings provide a simplified method to examine tuberisation in *Plectranthus esculentus*, much the same as in potato.
- Good results were obtained with both two-node and single-node cuttings taken from induced plants.
- Exposure to inductive conditions are required in order to induce tuberisation on cuttings.
- It is best to express the results of tuberisation trials in terms of the percentage of the

cuttings that show signs of tuber induction (swollen underground shoot bases and tubers). This is in agreement with the accepted practice in analysis of induction in potato cuttings (Ewing, 1985).

- Differences in tuberisation on the cuttings could be due to the position of the cutting on the stem of the plant, and so be a function of the age of the underground bud. The effect of bud age as well as leaf age should be experimentally tested.

References

BEYERS, E.A., HAMMES, P.S. & NEL, P.C., 1976. Doeltreffendheid van fitotronfasiliteite vir landbounavorsing. *Gewasprod.* 5, 37 - 40.

BODLAENDER, K.B.A., 1963. Influence of temperature, radiation and photoperiod on development and yield. Pp. 199 - 210 in: Growth of the potato.Edited by J.D. Ivins and F.L. Milthorpe. Butterworths, London.

BODLAENDER, K.B.A., 1981. The role of nitrogen in different periods of the development of the potato. *Potato Res.* 24, 221 - 222.

CHAPMAN, H.W., 1958. Tuberization in the potato plant. *Physiologia Plantarum* 11, 215 - 224.

COX, A.E., 1967. The potato a practical and scientific guide. W.H. & L. Collingridge, London.

DE VRIES, H., 1878. Beiträge zur speziellen physiologie landwirchaftloicher kulturpflanzen. IV. Keimungsgeschichte der kartoffelknollen. *Landwirtsch. Jährb.* 7, 217 - 249. (As quoted by Ewing, 1985).

EWING, E.E., 1976. Use of potato cuttings to study 'tuberization stimulus' in *Andigena* populations. *Am. Potato J.* 53, 358 - 359.

EWING, E.E., 1978. Critical photoperiods for tuberization: a screening technique with potato cuttings. *Am. Potato J.* 55, 43 - 53.

EWING, E.E.,1981.Heat stress and the tuberization stimulus. *Am. Potato J.* 58, 31 - 49.

EWING, E.E., 1985.Cuttings as simplified models of the potato plant. Ch. 5 in: Potato physiology. Edited by P.H. Li. Academic Press, Orlando.

EWING, E.E. & STRUIK, P.C., 1992. Tuber formation in potato: induction, initiation and growth. *Hort. Rev.* 14, 89 - 198.

EWING, E.E. & WAREING, P.F., 1978. Shoot, stolon and tuber formation on potato (*Solanum tuberosum* L.) cuttings in response to photoperiod. *Pl. Physiol.* 61, 348 - 353.

GREGORY, L.E., 1956. Some factors for tuberization in the potato plant. *Am. J. Bot.* 43, 281 - 288.

GREGORY, L.E., 1965. Physiology of tuberization in the potato plant (tubers and tuberous roots). Pgs. 1328 - 1354 in: Encyclopedia of plant physiology Vol 15/1. Edited by W. Ruhland. Springer-Verlag, Berlin.

HAMMES, P.S., 1971. Tuber initiation in the potato (*Solanum tuberosum* L.). *Agroplantae* 3, 73-74.

HAMMES, P.S. & BEYERS, E.A., 1973. Localization of the photoperiodic perception in potatoes. *Potato Res.* 16, 68 - 72.

HAMMES, P.S., BEYERS, E.A. & NEL, P.C., 1973. Fitotronfasiliteite vir landbounavorsing. Tegniese mededeling No. 13. Departement Landbou Tegniese Dienste, Pretoria.

HAMMES, P.S. & NEL, P.C., 1975. Control mechanisms in the tuberization process. *Potato Res.* 18, 262 - 272.

ISBELL, C.L., 1931. Regenerative capacities of leaf and leaflet cuttings of tomato and of leaf and shoot cuttings of potato. *Bot. Gaz. (Chicago)* 93, 192 - 201. (As quoted by Ewing, 1985).

KAHN, B. & EWING, E.E., 1983. Factors controlling the basipetal pattern of tuberization in induced potato (*Solanum tuberosum* L.) cuttings. *Ann. Bot.* 52, 861 - 871.

KAHN, B.A., EWING, E.E. & SENESAC, A.H., 1983. Effects of leaf age, leaf area, and other factors on tuberization of cuttings from induced potato (*Solanum tuberosum*) shoots. *Can. J. Bot.* 61, 3193 - 3201.

KNIGHT, T.A., 1806. On the inverted action of the albunomous vessels of trees. *Philos. Trans. R. Soc. London* 96, 293 - 304. (As quoted by Ewing, 1985).

KNIGHT, T.A., 1809. On the origin and formation of roots. *Philos. Trans. R. Soc. London* 96, 169 - 176. (As quoted by Ewing, 1985).

KRAUS, A., 1978. Tuberization and absisic acid content in *Solanum tuberosum* as affected by nitrogen nutrition. *Potato Res.* 21, 183 - 193.

KUMAR, D. & WAREING, P.F., 1972. Factors controlling stolon development in the potato plant. *New Physiol.* 71, 639 - 648.

KUMAR, D. & WAREING, P.F., 1973. Studies on tuberization in *Solanum andigena*. II. Growth hormones and tuberization. *New Phytol.* 73, 833 - 840.

KUPFER, E., 1907. Studies in plant regeneration. *Mem. Torrey Bot. Club* 12, 195 - 241. (As quoted by Ewing, 1985).

MADEC, P. & PERENNEC, P., 1962. Les relations entre l'induction de la tubérisation et la croissance chez la plante de pomme de terre (*Solanum tuberosum* L.). *Ann. Physiol. Veg.* 4, 5 - 84. (As quoted by Ewing, 1985).

MARINUS, J. & BODLAENDER, K.B.A., 1975. Response of some potato varieties to temperature. *Potato Res.* 18, 189 - 204.

MENDOZA, H.A. & HAYNES, F.L., 1977. Inheritance of tuber initiation in tuber bearing *Solanum* as influenced by photoperiod. *Am. Potato J.* 54, 243 - 252.

MENZEL, C.M., 1985. The control of storage organ formation in potato and other species; A review. Part 1. *Fld. Crop Abstr.* 38, 527 - 537.

SALISBURY, F.B. & ROSS, C.W., 1978. Plant physiology. Wadsworth Publishing Company, Belmont.

STEER, B.T., 1980. The bulbing response to daylength and temperature of some Australian onion (*Allium cepa* L.) cultivars. *Australian J. Agric. Res.* 31, 511 - 518.

VAN DER ZAAG, D.E. & VAN LOON, C.D., 1987. Effect of physiological age on growth vigour of seed potatoes of two cultivars. 5. Review of literature and integration of some experimental results. *Potato Res.* 30, 451 - 472.

VÖCHTING, H., 1887. Über die bildung der knollen. *Bibl. Bot.* 4, 1 - 55. (As quoted by Ewing, 1985).

VREUGDENHIL, D. & STRUIK, P.C., 1989. An integrated view of the hormonal regulation of tuber formation in potato *(Solanum tuberosum)*. *Physiologia Plantarum* 75, 525 - 531.

WELLENSIEK, S.J., 1929. The physiology of tuber formation in *Solanum tuberosum* L.

Mededelingen Landbouwhogeschool, Wageningen 33, 6 - 42.

WENT, F.W., 1959. Effects of environment of parent and grandparent generations on tuber production by potatoes. *Am. J. Bot.* 46, 277 - 282.

WERNER, H.O., 1935. The effects of temperature, photoperiod and nitrogen level upon tuberization in the potato. *Am. Potato J.* 12, 274 - 280.

WERNER, H.O., 1954. Anomolous tuberization of *Solanum tuberosum*. *Am. Potato J.* 31, 375.

WOOLLEY, D.J. & WAREING, P.F., 1972a. The role of roots, cytokinins and apical dominance in the control of lateral shoot formation. *Planta* 105, 33 - 42.

WOOLLEY, D.J. & WAREING, P.F., 1972b. The interaction between growth promotors in apical dominance. *New Phytol.* 71, 781 - 793.