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ABA concentration in shoot tips of *Amelanchier alnifolia* during the growing season and in relation to micropropagated plantlets

by

Susan A. Ramsey

A Thesis Submitted to

the Faculty of Graduate Studies

University of Manitoba

In Partial Fulfilment of the

Requirements for the Degree

of Master of Science

Department of Plant Science

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ABA CONCENTRATION IN SHOOT TIPS OF *Amelanchier alnifolia* DURING THE GROWING SEASON AND IN RELATION TO MICROPROPAGATED PLANTLETS

BY

SUSAN A. RAMSEY

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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There are a number of people who have contributed to the project in one way or other and who I would like to thank: to Lwellyn Armstrong of the Statistical Advisory Service for helping me design the nested ANOVA model and analyze the data, and to the people of the Horticulture lab for their friendship, support, and good humour.

Lastly, I would like to thank my husband Doug for his help on the computer, both to generate the figures, and to format this document. To my son Steven, I thank for being as patient as a child can be and a promise that we will get to Disney World yet.

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ABSTRACT

Ramsey, Susan A. M.Sc. Department of Plant Science, University of Manitoba. ABA concentration in shoot tips of *Amelanchier alnifolia* during the growing season and in relation to micropropagated plantlets. Major Professor: Dr. W.R. Remphrey.

Abscisic acid (ABA) concentrations were investigated in relation to orchard-grown shoots of *Amelanchier alnifolia* Nutt. (saskatoon) over the course of one growing season, in explants before culture, and in shoots and plantlets produced in culture. The ABA seasonal trend of *A. alnifolia* was related to patterns of shoot growth and development. Neoformed shoot expansion corresponded to decreasing ABA concentrations and shoot growth stopped around the time of a mid season peak in ABA. Vegetative maturity also coincided with this mid season peak. Leaf primordium production in the bud continued to be active for several weeks past vegetative maturity even though the bud itself was not able to break and regrow.

ABA concentrations were measured in explants before culture and then at the end of the multiplication and rooting stage, for three dates. Significant differences existed in ABA concentrations of shoot tips excised at various times during the growing season but by the end of the multiplication stage, ABA content was similar. At the end of the rooting stage, there were differences in plantlet ABA concentrations between dates and more ABA was found in non rooted plantlets than in rooted ones. ABA was of limited use as an indicator of performance under the experimental conditions tested and there was only a weak relationship between increased ABA and decreased plantlet quality. Variation in ABA in both shoots and plantlets produced *in vitro* could be explained to some extent by jars used at the multiplication stage and not explants, but much of the

difference could not be accounted for by the variables tested.

FOREWORD

This thesis has been written in manuscript style. Chapter 1, entitled "The relationship of ABA concentrations to morphological and developmental features of *Amelanchier alnifolia* shoots" has been submitted to the Canadian Journal of Botany. Chapter 2, entitled "ABA concentrations in shoot tip culture and their relation to culture date and stage in culture of *Amelanchier alnifolia*" has not yet been submitted for publication.

INTRODUCTION

Amelanchier alnifolia Nutt. (also known as saskatoon on the Canadian prairies) is a native fruit bearing shrub that has been valued for its flavourful berries since the presettlement of western Canada. More recently, several cultivars of *A. alnifolia* have been put into commercial production and appear to have considerable potential as a cultivated fruit crop. *A. alnifolia* is well adapted to the harsh climatic conditions of the prairie provinces and as a result this plant could significantly contribute to the diversification and health of the prairie agricultural economy by enhancing alternative agricultural production and by promoting the development of mixed farming operations.

Increased commercialization of *A. alnifolia* necessitates that certain production problems must be overcome, in particular the difficulty in obtaining a sufficiently large quantity of plants to expand orchards. Unfortunately, it has been a challenge to propagate *A. alnifolia* by traditional methods such as cuttings, budding, or suckers. In addition, there is a need for genetic uniformity of plants so that berries mature and ripen more evenly and allow mechanical harvesting to occur. Expansion of the industry means more acreage will be planted and put into production and mechanical harvesting is the only option for an efficient and economical means of berry collection in large orchards.

Research suggests that micropropagation, an asexual form of propagation which is done under aseptic conditions, has the potential to assist the commercialization of *A. alnifolia* by producing a rapid and steady supply of genetically uniform plants. However, *A. alnifolia* is still a relatively new crop and, although there have been studies done on various aspects of its biology as well as improvements made to its propagation, the

information base is not as extensive as for other well established fruit crops like apple and strawberries. Therefore, more research is needed and specific questions could be addressed, such as assessing the relationship of vegetative growth to micropropagation.

Vegetative propagation success has been shown to vary in relation to stages in the growth cycle and time of year. Abscisic acid (ABA) is a plant growth regulator that has been linked to seasonal cycles, and changes in ABA concentrations have been associated with plant growth, cessation of growth, and dormancy in temperate woody plants. Therefore, there may be a relationship between ABA and micropropagation success. Moreover, there is some evidence that ABA has a role *in vitro* in inhibiting growth or affecting the capacity of the explant to produce shoots and roots, but the influence of ABA in the micropropagation of *A. alnifolia* and on the performance of the subsequent plantlets has not been established. Therefore, this thesis has two main areas of research: the first will monitor ABA concentrations in orchard-grown *A. alnifolia* throughout the season and determine the relationship of ABA to various aspects of shoot and bud development which will provide background information that can be used as a comparison for *in vitro* ABA concentrations. The second area will focus on ABA in the context of micropropagation to ascertain if differences in explant ABA taken at various times in the growing season are retained throughout culture and have an effect on the morphological features produced by the plantlets and on rooting.

LITERATURE REVIEW

Amelanchier alnifolia

Distribution and Origins

Amelanchier alnifolia Nutt. belongs to the Rosaceae family, subfamily Pomideae. There are several other pome fruit bearing members of this subfamily which include *Sorbus* (mountainash), *Crataegus* (hawthorn), *Malus* (apple), *Pyrus* (pear), *Cotoneaster*, and *Aronia* (chokeberry) (Griffiths 1984).

A. alnifolia is native to the Canadian Prairies, and is found naturally from Alaska, the Yukon, and the Northwest Territories, to the northwestern and northcentral United States (Williams 1994). The habitat for *Amelanchier* ranges from wet to dry and from non-calcareous to highly calcareous soils (Miller and Stushnoff 1970). Some species are found in dry, rocky areas which suggests that there may be some drought resistance (Miller and Stushnoff 1970). The only soils in which *Amelanchier* does not grow are poorly drained or heavy soils lacking humus (Harris 1989).

In western Canada the common name for *A. alnifolia* is saskatoon. The name came from the plains aboriginals who found an abundance of these fruit-bearing shrubs in the vicinity of what is now known as Saskatoon in Saskatchewan. The plants were called "mis-sak-qua-too-min" by the Cree which later became shortened to saskatoon (Turner and Szczawinski 1979).

One of the original saskatoon products was pemmican. The berries were stirred into a boiling mixture of pulverized deer or buffalo meat, cooled, and moulded into cakes. The aboriginals used pemmican as the major winter storage food and for hunting and war

rations. Early settlers and Arctic explorers adopted the aboriginals' methods of preserving dried and semi-dried saskatoon, thus developing a taste for the berries which are still enjoyed today.

Saskatoon was first cultivated on the Canadian prairies in 1918 at the Agriculture Canada Research Station, Beaverlodge, Alberta, by W. D. Albright (Harris 1976). Albright planted a hedge of wild saskatoon from pieces of root "crowns" that had been dug up during land clearing. Selection did not begin until several years later when Albright noticed that fruit from different plants did not taste the same and as a result some 27 selections were made (Harris 1976). In addition to Albright, work at Beaverlodge was also carried out by John Wallace who evaluated numerous seedlings from the best plants and then made selections from those. The culmination of the work was the formal release in 1952 of the two best cultivars that Wallace named 'Pembina' and 'Smoky'. A few years later Beaverlodge also released 'Forestburg' and 'Altaglow'. To date, there are several other cultivars suited to Canadian conditions and commercially available. These include 'Bluff', 'Buffalo', 'Honeywood', 'Martin', 'Moonlake', 'Nelson', 'Northline', 'Paleface', 'Pearson 2', 'Sturgeon', and 'Thiessen' (Williams 1994).

Processing and marketing of saskatoon began in the 1970s through the cooperative efforts of Alberta Agriculture, Agriculture Canada, and an Alberta growers group called the Peace Country Small Fruit Growers' Society (Mazza 1989). They developed and test marketed a specialty gift pack containing saskatoon jelly, chokecherry jelly, rose hip jelly, and red clover honey. The popularity of saskatoon increased and soon other products like

syrup, wine, liqueur, juice, ice cream toppings and pie fillings, as well as fresh and frozen berries, were developed.

Economic Value

There are approximately 607 ha of saskatoon planted in the three Prairie provinces (Williams 1994). In Saskatchewan there are an estimated 162-243 ha, 61 of which are in production, while Alberta has between 243 and 405 ha, with 121 of these in production. In Manitoba there are approximately 40 ha saskatoon.

Although it takes 7 to 8 years for saskatoon to reach full production, the average crop yields 3,300 to 4,500 kg/ha for 30-50 years (St. Pierre 1991b). Optimal conditions can produce yields of 6,700 kg/ha (St. Pierre 1991b). Berry prices can vary depending on how the fruit was harvested. Williams (1994) reported that prices for berries harvested by hired pickers ranged from $5.10-$6.60/kg while U-pick operations charged $3.55-$4.40/kg. Machine harvested berries should sell at the same price as those harvested by hired pickers if the fruit is not damaged and is of comparable ripeness (Williams 1994).

Harvesting Options

There are several methods of harvesting berries, which include: U-pick, hired manual pickers, hand-held harvesters, and a range of mechanical harvesters as described by Williams (1994). U-pick operations are generally smaller operations and tend to be located near large population centres. Large acreages may market on a U-pick basis when the plants first come into production and the yield is not large enough to justify the cost

of a mechanical harvester. Hired pickers may also be used in similar ways. Pickers can harvest an average of 2-4.5 kg of berries per hour.

A semi mechanical harvester can be five to six times more efficient than hand harvesting (Mahadeva 1985; Williams 1994). Also, less than three percent of the harvested fruit would be bruised and this would permit more fresh fruit marketing (Mahadeva 1985; Williams 1994). The only disadvantages of the hand-held harvester include the need for additional cleaning of the fruit and the possibility of twig damage to the plants (Williams 1994).

There are two types of mechanical, over-the-row harvesters: self-propelled and tractor pulled. A mechanical harvester can harvest 0.4 ha per hour and is really the only option for growers who have large orchards, or who want a back up system in case they cannot hire enough pickers or do not attract enough U-pick customers (Williams 1994). However, proper row spacing and pruning are essential to accommodate the use of mechanical harvesters and minimize the degree of crop loss and damage to the bushes. There are some disadvantages associated with mechanical harvesting, one of which is the number of insects that are shaken from the plants and contaminate the harvested fruit. Similarly, diseased berries, especially those with grey mold, drop with the other fruit and contaminate the harvested berries. Lastly, machine harvested fruit deteriorates more quickly than hand harvested fruit and must be cooled or frozen as quickly as possible.

Morphology and Sexual Reproduction

A. alnifolia is a deciduous shrub that can range in height from 1 to 4 m. *A. alnifolia* most often is multiple-stemmed, low and spreading although it can be erect and slender depending on cultivar and growing conditions (Griffiths 1984). The leaves are simple, alternate, stipuled and petioled, obtuse or rounded (Griffiths 1984). There are generally 15 teeth to a side, with the teeth being most evident toward the distal end of the leaf (Williams 1994). The flowers are described as perfect, regular, and in racemes terminating in short leafy branches. There are 7-12 white flowers which make up an inflorescence (St. Pierre and Steeves 1990). Each flower consists of 5 petals, 5 sepals, 10-20 stamens, 2-5 styles (free or united at the base or to the middle), and a compound ovary with 2- 5 carpels (Fig. 1a). Each carpel is nearly divided by a false partition and forming a 4-10 loculated pome fruit with one ovule per locule (Fig. 1b). The carpel walls are firm and the pome is small, mealy or juicy and purple in colour.

The production of fruit and seed requires two seasons of development and growth (St. Pierre and Steeves 1990). The floral bud forms in the first season and in the second season anthesis and fruit development occur. The sequence of anthesis begins with the terminal flower and basal three to four laterals opening first followed by those in the midposition (Steeves and Steeves 1990). The mean period of anthesis is 14 days and flowers last anywhere from 2 to 5 days until petal drop (St. Pierre and Steeves 1990).

A. alnifolia berries are mature approximately 38 days after petal drop (Olson and Steeves 1982) and the ratio of mature fruit to ovary-bearing flowers is low (St. Pierre 1989). Insects and frost that cause damage to flowers and immature fruit are suspected

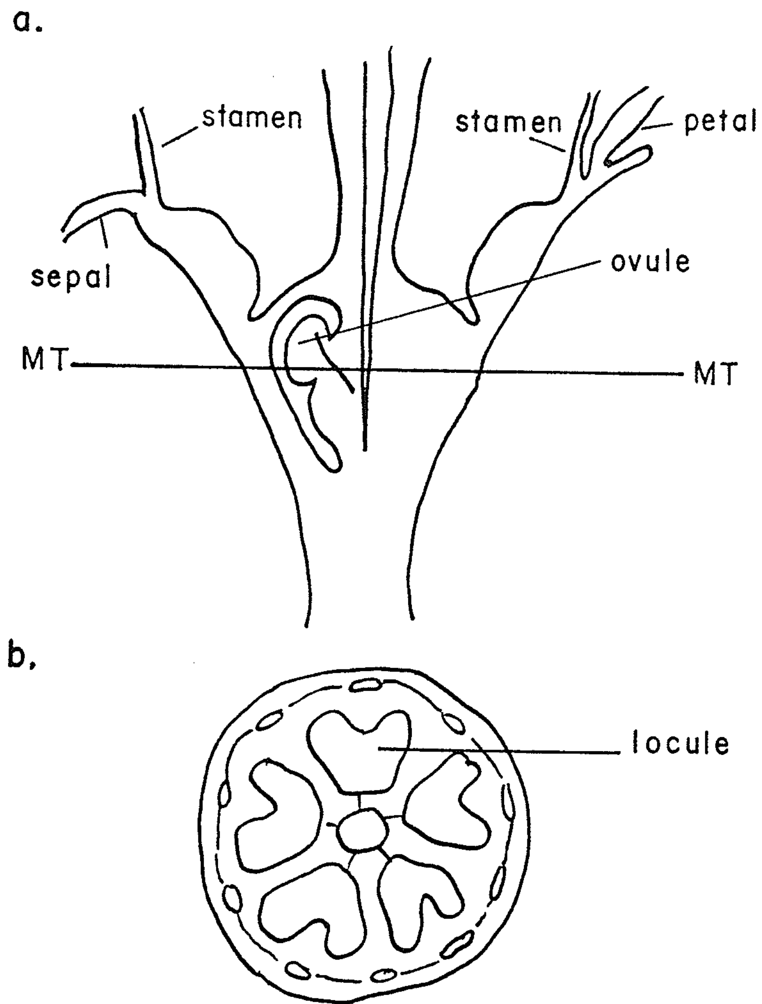


Fig. 1 a and b. Diagram of the inferior ovary of *A. alnifolia* (after Olson and Steeves 1982). The two ovules normally present in each locule are not illustrated. Fig. 1a. Longitudinal view of the inferior ovary. Fig. 1b. Median transection of ovary at the level of line MT in Fig. 1a.

to be the primary reasons for a low mature fruit:flower ratio (St. Pierre 1989). Earlier studies done by Olson and Steeves (1983) also found that floral tissues were vulnerable to frost and that massive injury occurred in the stylary, placental, and ovular tissue regions of both blossoms and immature fruits.

The short flower longevity suggests that *A. alnifolia* is a self-compatible species with decreased levels of outcrossing (St. Pierre and Steeves 1990). Olson (1984) demonstrated that autogamy (i.e., transfer of pollen to the stigma from the anthers in the same flower) is possible without insects due to the proximity of the anthers to the stigmas just prior to anthesis in conjunction with the preanthesis shedding of pollen. St. Pierre (1991a) also stated that insects do not appear to be necessary as pollen carriers of *A. alnifolia*, although flowers exhibit pollen and a stigmatic exudate which could contribute to overall attractiveness to insects (Olson 1984).

Propagation

At present, demand for the fruit of saskatoon exceeds the supply (St. Pierre 1992). The expansion of the saskatoon industry requires that propagation must take into account not only the need to obtain plants on a sufficiently large scale (Harris 1976; St. Pierre 1992) at reasonable prices (Nelson 1987; Ronald 1989) but the need for uniform plants suitable for machine harvesting (Casement 1976; Pruski *et al.* 1990). There have been several methods used to propagate saskatoon, some of which include: seed, softwood cuttings, hardwood cuttings, root cuttings, budding onto a rootstock, propagation from suckers (division of crowns), and micropropagation. However, not all forms of

propagation can be used to commercially satisfy the requirements of an expanding industry.

Seeds

Seed propagation has been the most widely used method (Griffiths 1984), however, the seedlings can show variation of up to 20-30% (St. Pierre 1991b) and this would be a disadvantage for machine harvesting. Different maturities, shapes, and quality levels make machine harvesting almost impossible (Henkes and Dietz 1992) and, therefore, the use of seed propagated plants would not be recommended for developing the saskatoon industry. Nevertheless, some variation may be desirable in terms of offering some resistance to disease or insect attacks (Cumming 1976).

Another problem encountered when using seed is the control of fungal growth during propagation (McTavish 1986). Fungal infestation occurs during cold stratification and spreads quickly if the stratification medium does not contain fungicide or is not pasteurized (McTavish 1986).

Softwood Cuttings

Softwood cuttings, cuttings prepared from soft and succulent new spring growth, have been rooted in outdoor mist frames but have produced only fair results at Morden, Manitoba (Cumming 1976). Rooting percentages ranged from a low of 12% to 36% which is far below what would be considered commercially satisfactory (Cumming 1976). Conversely, Wallace and Graham (1976) reported that over 90% of softwood cuttings

taken in late May in Washington D.C., and southern Ontario rooted within a month. Casement (1976) reported that cuttings are not difficult to root, but the problem was getting the rooted cuttings to survive the first winter.

One of the most important considerations in obtaining high rooting percentages of softwood cuttings is date of collection (Bishop and Nelson 1980). They found that cuttings taken as late in the season as possible, but while the plant was still in active growth, produced the highest rooting percentages.

Rooting is also affected by cutting length and propagation environment. Bishop and Nelson (1976) reported that the larger cutting length had better rooting percentages and that the best rooting percentages and root mass occurred when the cuttings were placed in a polyethylene-covered bed or when bottom heat was used.

Indolebutryric acid is often applied to softwood cuttings. However, rooting hormones were found by Bishop and Nelson (1976) to have no significant effect on rooting percentages or root mass.

Etiolation is a beneficial aid to rooting softwood cuttings of saskatoon. Nelson (1987) found that if bushes in the field were cut back (rejuvenated), grown in black polyethylene tents, and regreened slowly, the cuttings rooted well. In two cultivars tested, the regreened etiolated treatments produced 95% rooting without auxin. However, a disadvantage to this technique is that stock plants need to be severely pruned to force strong cuttings and nursery growers should establish a stock block for the sole purpose of taking cuttings (Nelson 1987).

Hardwood Cuttings

Hardwood cuttings, that is mature shoots formed the previous season, have proven to very difficult to root (Harris 1976). Therefore, this method is not considered feasible for commercial production of saskatoon.

Root or Rhizome Cuttings

Root cuttings are a recommended form of propagation. However, Griffiths (1984) suggests that the underground tissue being used is actually rhizomatous but indicates that morphological and anatomical studies are needed to clarify the confusion. In any event, underground tissue has been shown to root readily (Harris 1976) and etiolated cuttings from these pieces have given excellent results at Morden (Cumming 1976).

There are several factors involved in successful propagation of root or rhizome cuttings. Griffiths (1984) concluded that cultivar affected regenerative capacity and that cuttings of larger diameter and length were superior to smaller cuttings. Conditions during propagation such as temperature, humidity, and medium may also influence propagation success.

A disadvantage to this method of propagation is that it can be difficult to obtain a large quantity of underground material from cultivars and their removal frequently damages the parent plant (Harris 1976).

Grafting

Grafting, including the use of budding (Hartmann and Kester 1983), has been used as a method of propagating saskatoon and is preferred over cuttings by some nursery growers (Bishop and Nelson 1976). *Malus* has been used as a rootstock but often resulted in early maturity occurring in mid summer. This maturity manifests in leaf discoloration and is likely caused by incompatibility (Nelson and Bishop 1976; Wallace and Graham 1976). Species of *Crataegus* and *Sorbus* have been tried as a rootstocks but, after five years, it was discovered that compatibility was extremely variable and growth of the rootstock became restricted (Bishop and Nelson 1976). *Cotoneaster* is the most promising rootstock for *Amelanchier* (Wallace and Graham 1976) and has produced a high degree of successful budding and superior nursery growth (Nelson and Bishop 1976). Determining the proper rootstock from within the various cotoneaster species has presented a problem as far as hardiness and transplantability are concerned (Wallace and Graham 1976). According to Wallace and Graham (1976) *Cotoneaster acutifolia* and *C. lucida* are two of the best understocks. Despite the apparent success, using the technique of budding for commercial propagation is questionable due to higher initial costs, rootstock suckering, and poor top growth (Ronald 1989).

Propagation from Suckers - Division of Crowns

"Suckers" are defined as shoots that arise from the periphery of an established plant and originate from underground stem tissue (Hartmann and Kester 1983). Suckers can be divided, and this procedure has proven to be an important method of propagation

because it is simple and reliable (Hartmann and Kester 1983). Cumming (1976) reported that suckers may be used to quickly multiply the number of plants. An average of 25 or more divisions per plant was reported for 8-year-old cultivar 'Success' while 5-year-old 'Honeywood' seedlings averaged 10 suckers (Ronald 1989). Suckers obtained by divisions grow well and the original plant makes a good recovery (Ronald 1989). Superior survival was observed when the stems of the suckers were either small in diameter and severely pruned, or large in diameter and lightly pruned (Griffiths 1984). The only disadvantage to this procedure is that commercial production depends on an available supply of vigorous stock plants (Ronald 1989).

Micropropagation

Micropropagation, also known as plant tissue culture or 'cloning' (Kyte 1983), is an asexual form of propagation which involves the cultivation *in vitro* of all plant parts under aseptic conditions (Torres 1989). Micropropagation has the potential to revolutionize the horticulture industry because it has: the capability to produce commercial quantities of plants in less time than conventional forms of propagation; the ability to grow more genetically uniform plants than by any other propagation means; and, the theoretical capacity to produce an infinite number of plants from one explant (Kyte 1983). Although the specifics vary by species, the general process of micropropagation involves several stages which Kyte (1983) outlines as: Stage I - the period of establishment; Stage II - the multiplication of shoots; and, Stage III - rooting (Fig. 2). If microcuttings are not rooted directly into soil, then a Stage IV is needed where

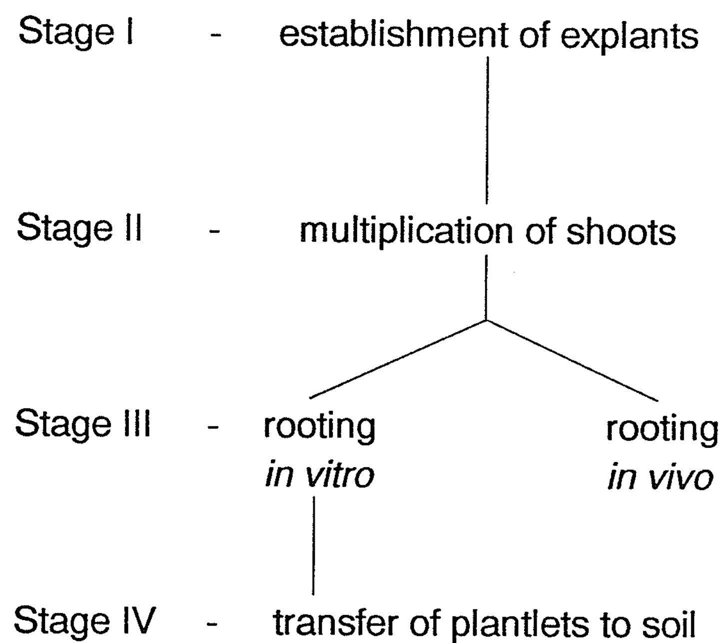


Fig. 2. Outline of the stages involved in the micropropagation of plants (*sensu* Kyte 1983).

rooted plantlets are transferred out of *in vitro* to a soil medium.

Micropropagation of saskatoon has the potential to meet the requirements of an expanding industry by producing a rapid and steady supply of plants with the uniformity needed to facilitate machine harvesting (Casement 1976; Pruski 1987). The main disadvantages are that it is time consuming and expensive.

The following is a review of literature on the micropropagation of *A. alnifolia*.

Explant - There have been various sources of explants utilized for micropropagating *A. alnifolia*: shoot tips, dormant buds, and active buds (buds in the silver tip stage, *sensu* Friesen 1986). Shoot tips were found to be the most satisfactory source of explant to use because they were easily sterilized and grew immediately after being placed on culture medium (Pruski 1987). Active buds produced mostly callus instead of shoots while dormant bud cultures showed contamination after incubation (Pruski 1987). Those dormant bud cultures that were not contaminated needed to have bud dormancy broken before shoot growth began.

Various cultivars of *A. alnifolia* have been used for shoot tip culture, including 'Thiessen', 'Northline', 'Pembina', and 'Smoky' (Pruski 1987). When shoot length and vigour were evaluated, Pruski (1987) found that 'Thiessen' and 'Northline' performed the best, producing shoots 29 mm or longer. Although all the shoot tips became established, the type of culture medium used had an influence on the growth of the explants.

Generally, larger explants grow and survive better than very small ones, but the elimination of pathogens becomes a greater problem with increased explant size (Seabrook 1980). Shoot tips used in micropropagation of *A. alnifolia* are usually excised

in lengths between 10 and 30 mm, then cleaned, sterilized, and trimmed to approximately 5mm for inoculation.

Media and Culture Vessels - The degree of success in initiating and growing plants *in vitro* depends on the type of medium used. There have been several formulations tried for *A. alnifolia*, including MS (Murashige and Skoog 1962), B5 (Gamborg *et al.* 1968), LS (Linsmaier and Skoog 1965), and Nc (Nitsch *et al.* 1968). Pruski (1987) found that explants established in MS and LS media produced shoots with lengths that were almost doubled to those grown in B5 or Nc. It was speculated that much higher nutrient levels, especially nitrogen in both MS and LS media might be responsible for the better establishment of cultures. When MS was used, the strength of the medium was adjusted according to the stage of culture. For example, 3/4 strength MS was used for the establishment stage while full strength was used for the multiplication stage and 1/3 strength for rooting (Harris 1985; Pruski 1987).

Size of the culture vessel was shown to affect *Amelanchier spicata* microshoot quality which in turn influenced rooting capacity (McClelland and Smith 1990). *A. spicata* explants responded to larger vessels by producing more shoots per explant and leaves with increased leaf area. McClelland and Smith (1990) suggest that shoots with larger leaf area are more likely to root, since rooting cofactors are produced in the leaves and are needed, in part, to control rooting capacity.

Plant Growth Regulators - Pruski (1987) showed that the concentration of BAP (benzylaminopurine) in the medium affected shoot length and the number of shoots per culture. He found that a BAP concentration of 8.88-13.3 μM was optimal for *A. alnifolia*

shoot multiplication (Fig. 2) and produced 8-10 shoots per explant with each shoot being 20 mm in length and having well developed leaves. A concentration of 17.6 μM BAP was reported by Harris (1985) to produce a maximum number of shoots. There were also differences in cultivar response to BAP concentration (Pruski 1987). At all concentrations, 'Thiessen' produced the most shoots as well as the longest shoots while 'Northline' produced the fewest and the shortest shoots of all the cultivars tested.

Auxins have been used to root *A. alnifolia in vitro* (Fig. 2), however there have been mixed results. Harris (1985) reported that occasionally there was up to 100% rooting of *in vitro* produced shoots when 0.1 mg L^{-1} of indoleacetic acid (IAA) was used. However, the percentage of rooted plantlets was more often less than 10%. Indole-3-butyric Acid (IBA) was used in preliminary experiments by Pruski (1987) who showed that the best rooting occurred when 2.45 μM of IBA was included in the MS medium. Pruski (1987) also noted that concentrations of IBA higher than 2.45 μM inhibited rooting as did BAP in the medium. A BAP concentration higher than 0.44 μM stopped shoots from rooting and concentrations between 2.22 μM and 6.65 μM produced shoots with callus formation only (Pruski 1987).

Culture Environment - *In vitro* cultures generally require a lower light intensity for the establishment and multiplication stages than for rooting (Fig. 2) (Murashige 1977). Pruski (1987) used an irradiance level of 55 $\mu E \cdot m^{-2} \cdot s^{-1}$ for both establishment and multiplication of shoots and then increased the level of irradiance to 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ for rooting. Increasing irradiance up to 70 $\mu E \cdot m^{-2} \cdot s^{-1}$ using white light produced three times the percentage of rooting shoots, two times the number of roots, and ten times the

length of roots per rooted plantlet compared to rooting under low light conditions of 10 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Pruski 1987). Rooting studies in which shoots were incubated for two weeks in the dark and then two weeks in the light (at 70 $\mu E \cdot m^{-2} \cdot s^{-1}$) did not produce roots but resulted in large amounts of callus (Pruski 1987). A 16 hour photoperiod was used for all stages of culture (Pruski 1987).

Temperature is also a factor in culturing *A. alnifolia.* Harris (1985) maintained temperatures at 23±2° C while Pruski grew cultures at a 24/22° C day/night temperature regime in a "Conviron" growth cabinet.

Rooting and Field Performance of Micropropagated Plants - Rooting success of micropropagated plantlets appears to be related to cultivar differences. It was shown that 'Smoky' was the easiest to root, with 63% of the shoots producing roots, while 53% of 'Thiessen' shoots rooted (Pruski 1987). Microcuttings produced at the end of the multiplication stage, may be rooted either *in vitro* or *in vivo* (Torres 1989). *In vivo* rooting eliminates the period of acclimatization of plantlets to greenhouse conditions as well as minimizing work and equipment. In addition, Pruski (1987) reported that microcuttings rooted directly in the greenhouse under non-sterile conditions often had survival rates of 100%. On the other hand, many plantlets rooted *in vitro* and transferred to a soil mixture did not survive and those that did often stopped growing until a new root system was developed (Pruski 1987).

Rooted micropropagated plantlets are eventually transferred from the greenhouse to the outdoors. Pruski (1987) and Struve and Lineberger (1985) reported that a 14-day period in the lath house before field planting reduced transplant shock and increased the

survival percentage of *in vivo* rooted plants to nearly 100%. These plants survived the winter and produced active shoots the following spring. Plants transplanted directly from the greenhouse to the field showed decreased percentage survival as well as reduced shoot length and number of shoots after 3 months (Pruski 1987).

Dormancy and Acclimation - Summer dormancy has been a problem with propagating *A. alnifolia*. As many as 80-90% of newly *in vivo* rooted plantlets stop shoot growth and go into dormancy by the time rooting is completed (Pruski 1987). Similarly, softwood cuttings are known to defoliate in the rooting beds and enter a dormant state (Bishop and Nelson 1980; Nelson 1987). Summer dormancy could be overcome and Pruski (1987) was able to get *in vivo* plantlets to resume growth with foliar sprays of GA_{4+7}, BAP, or a combined treatment. Cold treatment, exposure to temperatures of 0° to 2° C, and applications of GA also are effective in breaking dormancy of softwood cuttings (Grainger 1980).

When the transition is made from summer to winter dormancy, it is known as 'vegetative maturity' and is defined experimentally as the point where removal of leaves will no longer stimulate lateral bud break (Fuchigami *et al.* 1982). In *A. alnifolia*, vegetative maturity was found to occur early in the season and was a prerequisite for cold acclimation (Friesen and Stushnoff 1989). After vegetative maturity, acclimation occurred following exposure to cool temperatures regardless of the daylength (Friesen and Stushnoff 1989). This suggests that early onset of vegetative maturity may be an adaptation to environmental stress and may improve winter survival by preventing regrowth of shoots late in the season. If shoots regrew late in the season, there would not

be sufficient time for the shoots to acclimate and become cold hardy before the onset of freezing temperatures, and as a result there would be dieback or death of the plant the following spring.

In contrast to vegetative dormancy, flower bud dormancy in 'Smoky' and 'Pembina' was discovered to increase rapidly, reach different points of deep rest and, therefore, have different chilling requirements (Kaurin *et al.* 1984). Hardening of flower buds starts after exposure to temperatures below 0° C and then hardiness levels are correlated with outdoor temperature (Kaurin *et al.* 1984). Flower buds are hardy to -45° C until the middle of March and then hardiness gradually declines (Junttilla *et al.* 1983). Hardiness of unopened buds was closely correlated with bud moisture content and loss of hardiness was associated with petal expansion (Juntilla *et al.* 1983).

Abscisic Acid

Abscisic acid or ABA in higher plants is involved in developmental processes such as bud and seed dormancy, abscission, elongation growth, stomatal closure, root growth, fruit ripening, senescence (Addicott and Carns 1983). ABA is also implicated in the response of a plant to environmental stress (drought, frost, increased temperature, waterlogging, salinity and attacks by pests and pathogens) (Levitt 1980; Jones and Jones 1989). ABA and relatively few other phytohormones have the ability to elicit a diverse range of physiological responses during plant development (Hetherington and Quatrano 1991). The response of a plant cell or tissue to a phytohormone exhibits certain characteristics which include:

1) the capacity of the response to occur only at a specific developmental stage of a tissue
2) the correlation of the response to levels of the hormone (i.e., dose-response curve)
3) the ability of the response to vary in its sensitivity to the hormone and cause displacement of the dose-response curve (Hetherington and Quatrano 1991).

ABA shares some similarities with IAA and GA. All three have relatively low molecular weights, similar chromatographic properties, similar aqueous and lipid solubilities, and they are easily extracted with ether (Addicott and Van Steveninck 1983). It is thought that these similar chemical characteristics help explain why the three interact in so many plant functions, either reinforcing or counteracting each other (Addicott and

Van Steveninck 1983).

History and Structure of ABA

Historically, ABA was first associated with the abscission of immature cotton bolls. The effective compound was isolated from such bolls, chemically identified, and called "abscisin II" (Addicott and Carns 1983). Later research into the induction and maintenance of bud dormancy lead Cornforth *et al.* (1965) to isolate sycamore "dormin" in leaves, which they chemically demonstrated to be identical to "abscisin II". Dormin and abscisin II are now known as abscisic acid (ABA) or 3-methyl-5-(1'-hydroxy-4'-oxo-2',6', 6'-trimethyl-2'-cyclohexen-1'-yl)-cis, trans,-2,4-pentienoic acid) (Fig. 3).

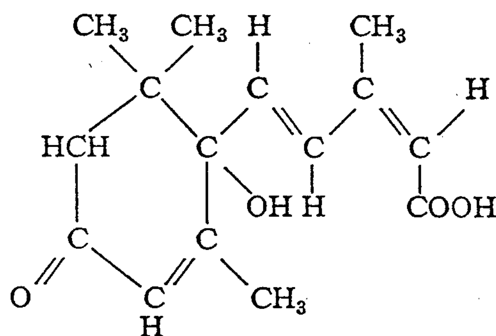


Fig. 3. Chemical structure of ABA.

Biosynthesis and Metabolism

Research on ABA biosynthesis focuses on two pathways: the direct pathway which involves the formation of ABA from a 15 carbon molecule (farnesyl pyrophosphate), and

the indirect pathway, which is the more accepted version, where ABA arises from mevalonic acid through the carotenoids (Fig. 4) (Creelman 1989). In the indirect pathway the sequence of events starts with a 5 carbon molecule and builds by increments of 5 carbon molecules to 10 carbons, 15 carbons, and up to 20 carbons. The condensation of two 20 carbon molecules forms a 40 carbon carotenoid which, when oxygenated, becomes violaxanthin (Creelman 1989). Subsequent oxidative cleavage of violaxanthin forms xanthoxin (Xan), a 15 carbon structure which is then converted into ABA (Creelman 1989). In the indirect pathway, one mole of ABA is synthesized from the cleaving of one mole of xanthophyll (Parry and Horgan 1992). However, the rate limiting step in ABA biosynthesis is not the oxidation of Xan to ABA but what occurs before Xan (Creelman 1989). Research done by Zeevaart and Creelman (1988) demonstrated that inhibitors of carotenoid biosynthesis, such as fluridone, reduced the carotenoid pool size and prevented accumulation of ABA. Mutants were also used to show that changes in carotenoid pool size are correlated with decreased ABA content and inability to accumulate ABA.

The initial ABA metabolites include ABA glucose ester, phaseic acid, 6'-hydroxymethyl ABA (Walton 1987). Walton (1987) also states that the major pathway of ABA metabolism has phaseic acid being further metabolised into dihydrophaseic acid, although, dihydrophaseic acid may not necessarily the end product of metabolism.

Metabolism and its roles, including activation and inactivation of hormonal activities and conversion of a hormone to storage and/or transport forms has been discussed by Walton (1987). Metabolites of ABA, when compared with ABA, have been found to vary in their ability to affect various physiological processes since almost any

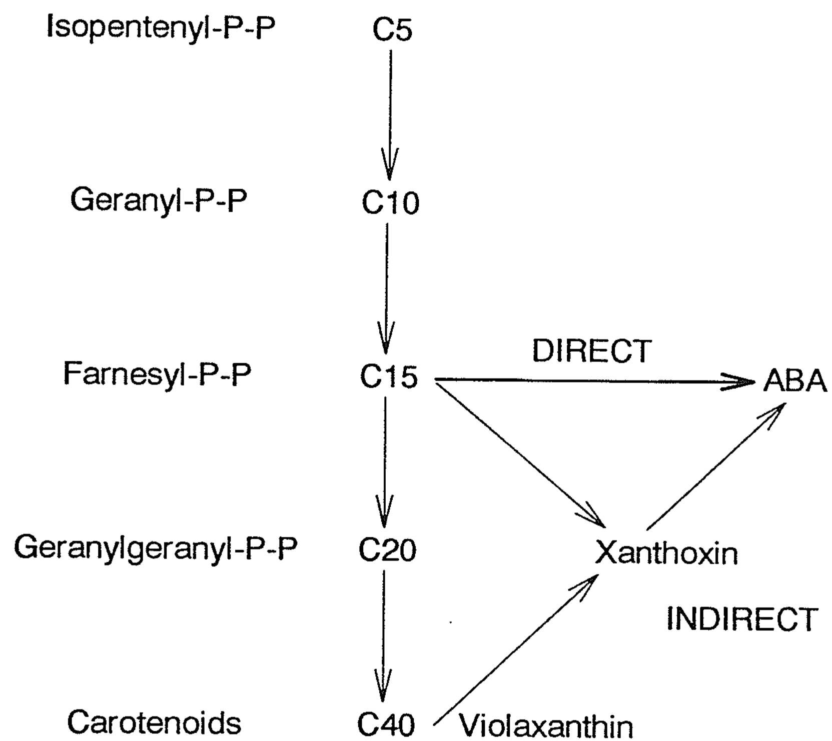


Fig. 4. Theoretical pathway of ABA synthesis in higher plants (after Creelman 1989). On the left is a generalized outline of the indirect pathway showing ABA being formed from carotenoids. The direct pathway for ABA synthesis has FPP as the branch point.

change in the ABA molecule reduces the apparent activity. The primary mode of ABA inactivation is thought to be dihydrophaseic acid which has shown no activity in any of the bioassays in which it has been tested. Lastly, storage and/or transport forms of ABA require that metabolites must be reconvertable to ABA, which include ABA glucose ester and 1'-glycoside of ABA, and not phaseic or dihydrophaseic acid.

Modes of Action

ABA has two modes of action which include slow and rapid responses (Addicott and Van Steveninck 1983; Hetherington and Quatrano 1991). Slow responses involve the synthesis of nucleic acids and proteins (Addicott and Van Steveninck 1983). Examples of such a response include the synthesis and secretion of α-amylase in germination, the synthesis of cellulases and pectinases in abscission, and proteases and hydrolases in senescence. Rapid responses affect membranes and include: reversible changes in K+ permeability in specialized cells such as guard cells and pullivini, changes in membrane regulated hydraulic conductivity of root cells, membrane changes that result in the release of hydrolytic enzymes, increased membrane integrity, and development of ion pump capacity that assists in osmoregulation (Addicott and Van Steveninck 1983).

Quantifying ABA

With the exception of ethylene, ABA is perhaps the easiest of the plant growth substances to analyze, and reliable measurements of ABA in plant extracts have been made for the last 20 years (Parry and Horgan 1991). ABA can be measured by physio-

chemical techniques such as gas/gas-liquid chromatography (GC and GLC), high performance liquid chromatography (HPLC), or more recently by immunoassays. Immunoassays are based on the competition of a known amount of sample antigen for a limited number of high affinity antibody binding sites (Weiler 1984).

The development of immunoassays using monoclonal antibodies has proved to be a rapid and sensitive method for measuring ABA in minute amounts of plant material (Walker-Simmons and Abrams 1991). The use of monoclonal antibodies has eliminated much of the variation in specificity (cross-reactivity) that was seen in polyclonal antisera due to multiple populations of antibodies (Ross *et al.* 1987). Monoclonal antibodies have been used in ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), and FIA (fluoroimmunoassay) (Walker-Simmons and Abrams 1991).

There are many advantages in using ELISAs. ELISAs are often less expensive to perform than RIAs and do not require radioactive tracers or specialized laboratory equipment (Walker-Simmons and Abrams 1991). In addition, ELISA methods are able to detect concentrations of ABA as low as 2×10^{-16} mol (Harris and Outlaw 1990) and show negligible cross reactivity with several ABA-related compounds (Norman *et al.* 1988).

ABA immunoassay methods need to be modified when any new tissue is assayed and then verified by independent ABA analysis using physico-chemical techniques (Walker-Simmons and Abrams 1991). Once an appropriate protocol has been established and validated, then very large numbers of samples can be assayed by an automated ELISA processor (Ross *et al.* 1987).

The Indirect ELISA

The most recent protocol for indirect ELISA was described by Walker-Simmons and Abrams (1991). A brief outline of the procedure includes:

1) add an ABA-4'-bovine serum albumin conjugate to the wells of a microtitre plate and incubate overnight at 4° C.

2) pipette (+)ABA standards or samples into test tubes. Add equal amounts of monoclonal antibody solution to each test tube, mix, and incubate overnight at 4°C.

3) wash the microtitre plate three times with washing buffer. Add the ABA standards with monoclonal antibody and the samples with monoclonal antibody to three replicate wells and incubate for 2 hours in the dark at room temperature.

4) wash the microtitre plate three times with washing buffer. Add rabbit anti-mouse alkaline phosphatase conjugate to each well, and incubate for 2 hours in the dark at room temperature.

5) wash the microtitre plate three time with washing buffer. Add p-nitrophenyl phosphate substrate to each well and incubate until the absorbance at 405 nm of control samples containing no ABA is approximately 1.0.

6) measure absorbance at 405 nm in an ELISA plate reader and stop the reaction by adding 50 µl of 5 N KOH.

The indirect ELISA immunoassay uses monoclonal antibodies specific to free cis,trans(+)ABA (Ross *et al.* 1987). There is no cross reactivity with cis,trans(-)ABA,

trans,trans(+)ABA, cis,trans(+)ABA-D-glucopyranosyl ester, cis,trans(+)ABA-cis-diol, xanthoxin, and all-trans-farnesol, and less than 0.1% cross reactivity with cis,trans(+)ABA-Me, phaseic acid, and dihydrophaseic acid (Ross *et al.* 1987).

Ross *et al.* (1987) indicate sources of error which lead to results that are less than satisfactory. One source of error includes storing standards with very low ABA concentrations for any length of time because ELISA estimates derived from "old" standards vary with position on the standard curve. Also the amount of purification required for each type of tissue, and at each stage of development for that tissue, must be determined before there is large scale use of the assay. Lastly, to prevent unexplained losses, or unusual variation in the ELISA, an internal standard may be used as part of the routine procedure for individual sample extracts.

Dormancy

Dormancy has evolved in perennial woody plants of the temperate zone as an adaptation to stress, allowing the plants the capacity to survive cyclically adverse microclimate conditions (Seeley 1990). The dormancy mechanism is suggested by Powell (1987) to exert a major influence on perennial woody plant growth and development throughout the year.

The annual shoot growth cycle begins in the spring with bud burst, which is thought to occur when the temperature is conducive for growth and the bud is free of any physiological dormancy (Powell 1987). Following bud break, there is a period of rapid shoot elongation after which elongation stops, resting buds are formed, and summer

dormancy develops, followed by winter dormancy (Fuchigami *et al.* 1982). In summer dormancy, dormant buds are unable to resume growth because of either the inhibitory effect of neighbouring organs (leaves, buds, etc.) or unfavourable environment, however, in winter dormancy, chilling temperatures are required before the bud can be induced to grow (Fuchigami *et al.* 1982). Once the chilling requirements are satisfied, and warmer temperatures of spring return, the annual cycle can begin again with the occurrence of bud burst (Powell 1987).

Dormancy and ABA

The classic work of Phillips and Wareing (1958) found that the inhibitor content of sycamore buds and leaves changed with the season, and that the period when dormant buds were developing coincided with increasing levels of inhibitors. Phillips and Wareing (1959) also examined the effect of short days on this inhibitor and reported that 2 short days were needed to increase the level of inhibitor in mature sycamore leaves, whereas apices needed 5 short days, before there was any marked effect of daylength on growth. Subsequently, Eagles and Wareing (1963; 1964) found a similar increase in the inhibitor content of birch leaves and buds under short days. In addition, they demonstrated that partially purified inhibitor could inhibit extension growth and induce dormant morphology even under a noninductive photoperiod. After the identification of abscisic acid (ABA), it was shown that continuous applications of ABA could induce the formation of dormant buds in birch, sycamore, and black currant (El-Antably *et al.* 1967). However, Lenton *et al.* (1972), who measured ABA concentrations by gas chromatography instead of

bioassays, found no increase in the ABA contents of leaves or buds of red maple, sycamore, and birch when these plants were transferred from long days to short days. Similarly, Hocking and Hillman (1975) did not find that leaf applied ABA induced bud dormancy in birch and alder growing in long days, and suggested that endogenous ABA in the leaves does not have a direct controlling effect on the formation of resting buds.

Despite these apparent contradictions to the role of ABA in dormancy, there have been many other studies that have found increased ABA concentrations during the development of dormancy. Some of these include: Wright (1975) with blackcurrant and beech buds, Webber *et al.* (1978) with shoots of Douglas-fir, Dumbroff *et al.* (1979) with maple buds and stems, Seeley and Powell (1981) with vegetative apple buds, Rogriguez *et al.* (1990) with hazel.

Dormancy and Other Hormones

It is not possible to say with absolute certainty that either the gibberellins or cytokinins play primary roles in the regulation of the dormancy mechanism (Powell 1987). Gibberellins increase with chilling but changes in gibberellin levels seem to be associated more with normal growth and development of the seedling than with the release of dormancy. In addition, exogenously applied cytokinins have been able to stimulate the growth of axillary buds under apical dominance, however it is not known if these substances are able to break resting buds.

ABA and Flowering

ABA does not have a central role in controlling flowering, although it does influence flowering in any of several ways, as described by Bornman (1983). In plants where flowering does not commence until after vegetative growth, applied ABA can accelerate the onset of flowering in some plants, but delay or inhibit flowering in others. In *Lupinus luteus*, ABA is involved in determination of the final morphology of the infructescence - ABA from the lower, first-developing fruits induces the abscission of flowers and young fruits higher on the stalk. However, in virus-infected plants, the developing basal fruits produced only 40 percent as much ABA as healthy fruits which permits the retention of more upper fruits and an elongate morphology of the infructescence.

CHAPTER 1

The relationship of ABA concentrations to morphological and developmental features of *Amelanchier alnifolia* shoots

ABSTRACT

Abscisic acid (ABA) concentrations were monitored in orchard-grown *Amelanchier alnifolia* Nutt. (saskatoon) shoots over the course of one growing season. The ABA concentrations dropped in the spring after bud break, had a mid season peak, and after a small decline, rose steadily toward the autumn. ABA concentrations were related to patterns of shoot growth and development. Neoform shoot expansion corresponded to decreasing ABA concentrations and shoot elongation stopped around the time of the mid season peak of ABA. Also coinciding with the mid season peak was the transition from summer to winter dormancy where lateral resting buds could no longer be stimulated to break following defoliation (vegetative maturity). Primordium production in the bud continued to be active for several weeks past vegetative maturity even though the bud itself was not able to break dormancy and regrow.

INTRODUCTION

Amelanchier alnifolia Nutt., commonly known as the saskatoon in western Canada, is a fruit bearing shrub which is gaining in importance as a commercial crop of the prairies (Steeves and Steeves 1990). There have been several studies of *A. alnifolia* which have addressed aspects of shoot development in stands of natural plants (Steeves and Steeves 1990; St. Pierre and Steeves 1990). These reports have shown that, although neoformation of shoots was observed by Steeves and Steeves (1990), most of the vegetative shoots were preformed and shoot and leaf expansion continued until approximately the end of May when a new terminal bud became visible (Fig. 5). (Neoformed shoots differ from preformed shoots in that the vegetative shoot is not entirely preformed in the resting bud and a portion of the leaves are formed during the growing season (Bell 1991)). Following shoot expansion, Steeves and Steeves (1990) reported that primordium initiation continued until mid-August for putative vegetative buds and later for those shoots which converted to the formation of inflorescences.

The term summer dormancy was adopted by Wareing (1956) to depict the stage of development when shoot growth stopped and resting buds were formed. During summer dormancy buds could be stimulated to break and prematurely expand, however, buds in winter dormancy were only able to resume growth after a period of winter chilling. The production of primordia ceased at the onset of winter dormancy.

Recently Fuchigami *et al.* (1982) developed a quantitative model for describing distinctive physiological stages of plant growth and development which included the concept of summer and winter dormancy. The term vegetative maturity was coined by

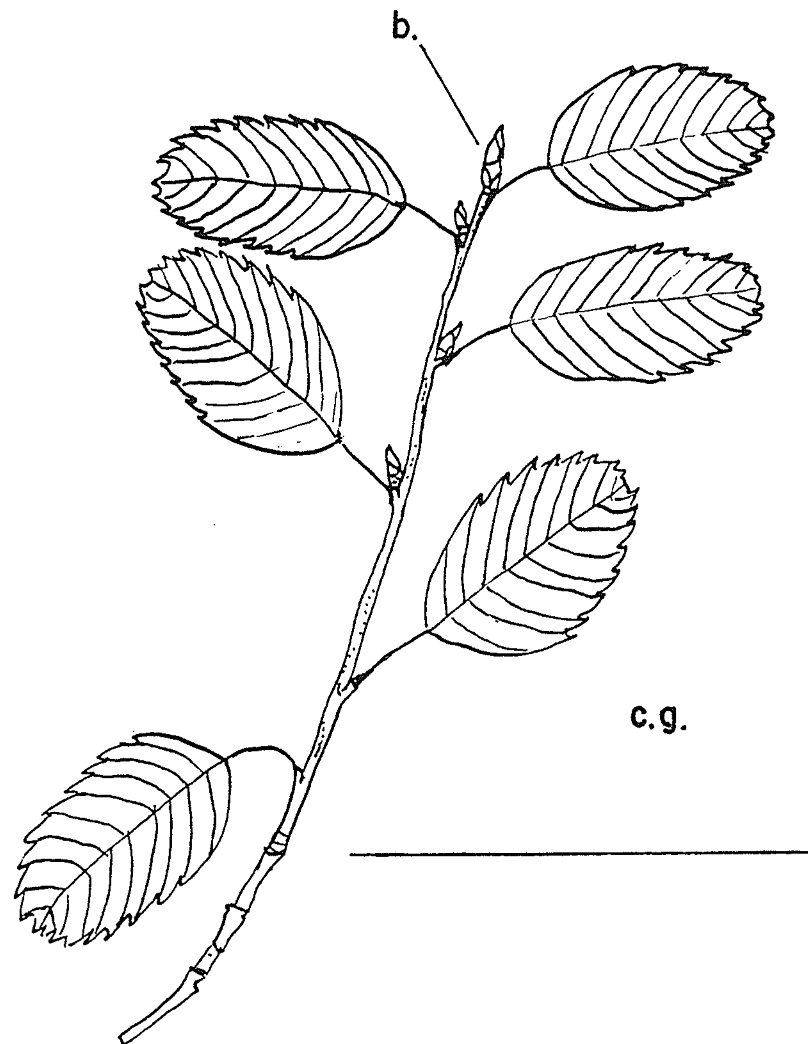


Fig. 5. Preformed vegetative shoot (after St. Pierre and Steeves 1990). b. terminal bud with cataphylls (bud scales). c.g. current year's growth - the expansion of a resting bud.

Fuchigami *et al.* (1982) and defined experimentally as the transition between summer and winter dormancy when dormant lateral buds could not be stimulated to break following defoliation. There have been studies of *A. alnifolia* which have addressed aspects of dormancy, vegetative maturity, and acclimation (Kaurin *et al.* 1984; Friesen and Stushnoff 1989). In these studies, vegetative maturity was needed before plants were able to acclimate and vegetative maturity was found to occur early. It is not clear, however, if the concept of vegetative maturity (*sensu* Fuchigami *et al.* 1982) corresponds with the termination of primordium production in the buds.

Endogenous growth inhibitors such as abscisic acid (ABA) have been implicated in the regulation of shoot development (Phillips and Wareing 1958, 1959; Vegis, 1964; Wareing and Saunders 1971; Wright 1975; Rodriguez *et al.* 1991). Rhythmic growth cycles have been correlated with fluctuating concentrations of ABA. Although there may be variation (Harrison and Saunders 1975), ABA concentrations are typically low at bud burst and gradually increase over the growing season. Some reports have indicated growth cessation and bud set are correlated with higher concentrations of ABA (Phillips and Wareing 1959; Seeley and Powell 1981; Rodriguez *et al.* 1991). The precise relationship of seasonal ABA concentrations to *A. alnifolia* shoot ontogeny has not been demonstrated.

As part of a larger study focusing on concentrations of ABA *in vitro*, the following study was undertaken to determine the relationship of ABA concentrations of orchard-grown *A. alnifolia* to aspects of bud development, shoot growth and its cessation, and vegetative maturity. In particular, the objective of this study was to examine the

relationship of vegetative maturity to primordium production patterns within buds and the relationship of ABA concentrations to stages of shoot and bud development.

MATERIALS AND METHODS

Plant Material

For this investigation, the cultivar 'Smoky' was used. The plants were located on a 0.4 ha site separated from an established commercial orchard 93 km south west of Winnipeg, Canada. The plants were entering their fifth year of growth.

Sampling and Measurements

Only adventitious shoot complexes (suckers) arising from the periphery of the established plants were sampled because the intent of the study was to follow the development of vegetative shoots (Fig. 6). Shoots on the parent plants could not be used because they had become reproductive. However, by the end of the growing season, even the previously vegetative shoots on the suckers were also becoming reproductive. Therefore, the study involved the analysis of sucker shoots which had converted to the reproductive state.

Shoots and Buds

Ten randomly selected shoots in various stages of elongation were collected weekly beginning May 15, 1992 and continuing to September 24, 1992. The shoots were transported back to the laboratory at the University of Manitoba in a cooler.

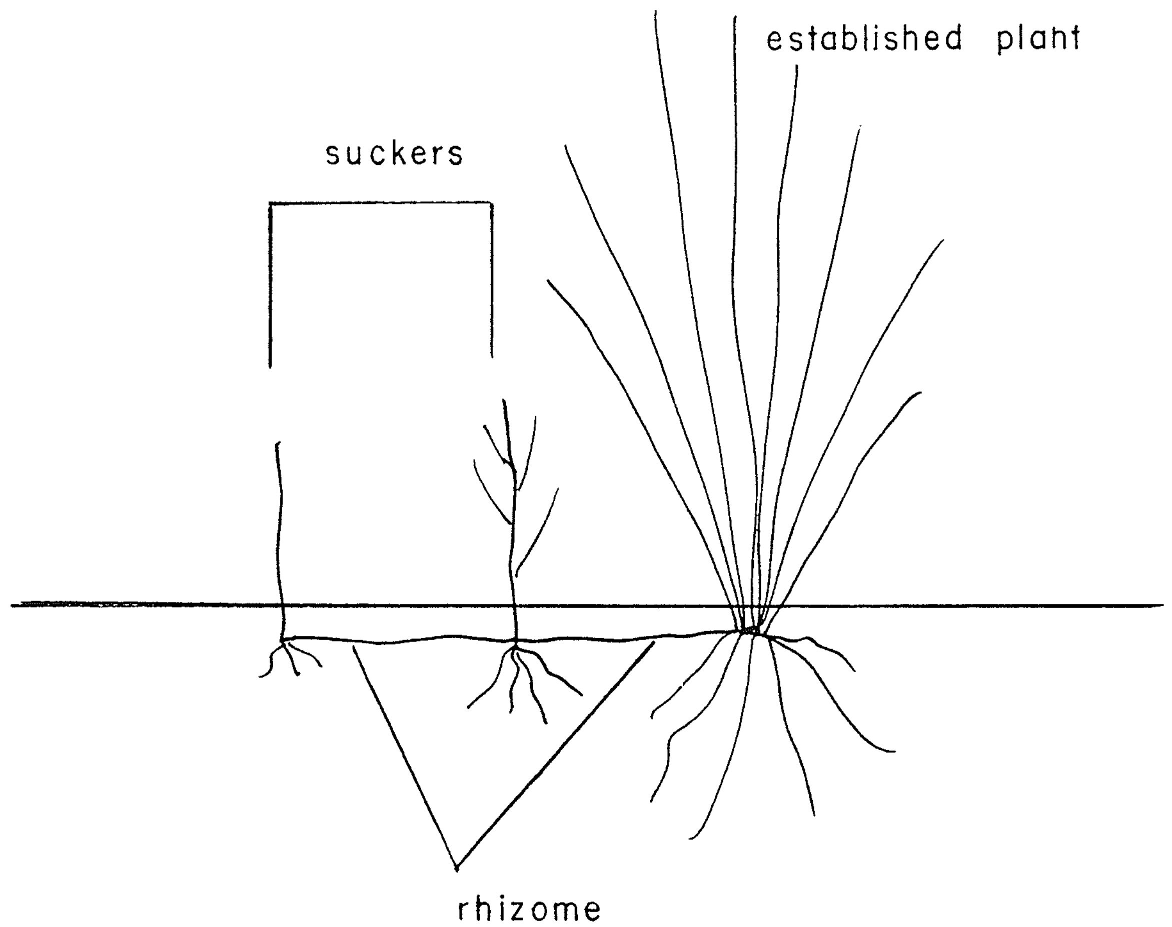


Fig. 6. A diagram representing the relationship of suckers to the established plant.

Measurements of shoot length (mm), the number of foliage leaves (opened and partially opened), and the number of primordia at the apex were recorded. The latter was obtained by dissection of the apical region of each shoot under a stereo microscope. The time of initiation of each leaf type (i.e., cataphylls, foliage leaves, floral bracts) was determined by direct observation of changes in the appearance of developing primordia. For verification, the number of cataphylls in the bud was compared to counts of fully expanded cataphylls shortly after the beginning of shoot growth and to cataphyll numbers determined in other studies of *A. alnifolia* (Steeves and Steeves 1990).

Vegetative Maturity

Vegetative maturity was determined by weekly tip removal and defoliation of ten randomly chosen vegetative shoots on plants in the field, starting June 5 and continuing to July 24. Each shoot was then monitored for 2 - 3 weeks for any changes in the bud. The date of onset of vegetative maturity was determined as the earliest date when defoliation no longer resulted in lateral bud burst, characterized by the emergence of green leaves from the bud, according to the definition of Fuchigami *et al.* (1982).

ABA Analysis

Four vegetative shoots were randomly selected biweekly and a length of stem including the upper three unfolded leaves was excised (Fig. 7), transported to the laboratory between damp newspaper in a cooler (Appendix A), and stored at -70° C. For processing, the individual samples were freeze-dried, ground, and 0.1 g freeze dry weight

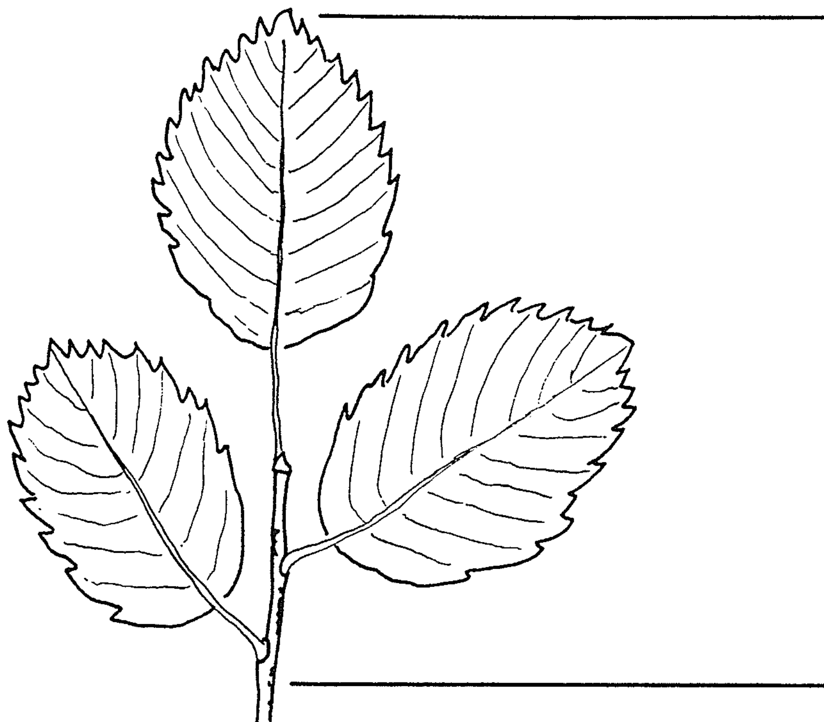


Fig. 7. Sketch of the upper three unfolded leaves which were used for ABA analysis.

was used as representative of that sample (except for the May 15 samples when the leaves were emerging from the bud and had a total dry weight of less than 0.01 g). Each sample followed the protocol established by Walker-Simmons (1987) for methanol extraction, resuspension, and ABA analysis by the indirect ELISA. The only deviations made to Walker-Simmons (1987) procedure were the elimination of internal standards during extraction, drying of the supernatant by nitrogen gas before resuspension, and a longer incubation time with p-nitrophenyl phosphate substrate. In addition, KOH was not used to stop the substrate incubation before the sample absorbance was measured.

Samples were stored at -70° C until there were enough for a tray. A test tray consisted of a maximum of 18 samples and a set of standards from 0 to 167 picograms of ABA. Each sample was diluted (the correct dilution was determined in preliminary trials) and then aliquotted into three wells of the tray. A linear regression was computed for each tray using the log of ABA standards against their absorbance, and an r^2 of not less than 0.94 was accepted (Fig. 8). The amount of ABA in the plant extract samples was based on the ABA standard curve and those samples that had ABA values falling within the linear range of the ABA standard curve were accepted while those samples that fell at either end of the curve were rejected. To calculate the ABA per gram of dry weight, the ABA value of each sample was multiplied by the dilution and then divided by 0.1 g which was the dry weight of each sample.

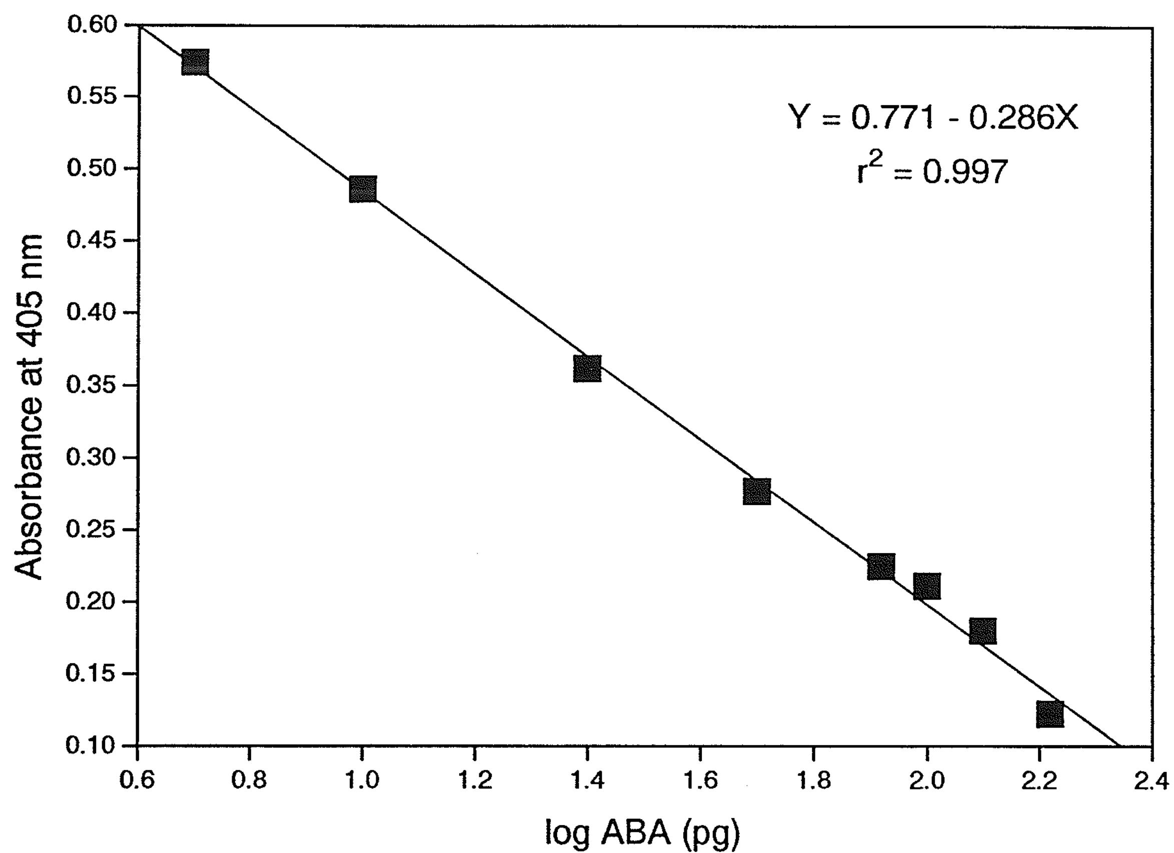


Fig. 8. Example of an ABA standard curve showing the measured ABA values (■) and the regression equation used to determine the ABA values of samples on a tray.

RESULTS

Seasonal Trend of ABA

When the leaves were first starting to open the mean ABA concentration (± standard error) was 1354±265 ng ABA (g dry weight)$^{-1}$ (Fig. 9). By June 5 there had been a considerable drop in ABA to 335 ng (g dry weight)$^{-1}$ followed by a gradual decline which continued to a season low of 125 ng ABA (g dry weight)$^{-1}$ on July 3. On July 17 there was a peak of 657 ng ABA (g dry weight)$^{-1}$ after which the concentration dropped to 280 ng (g dry weight)$^{-1}$ on July 31. There was a steady increase to 985 ng ABA (g dry weight)$^{-1}$ on September 24 when the leaves had changed colour.

ABA and Shoot Development

Shoot Elongation

Shoots started to expand by May 15 and elongation was almost linear with time until July 3 (Fig. 9). Expansion then slowed and had ceased by July 17. It was noted that this reduction in shoot elongation occurred around the same time that mean ABA concentrations began to increase. Shoot elongation stopped approximately at the time of the observed mid season peak. In general, it appeared that as the shoot elongated, mean ABA concentrations decreased.

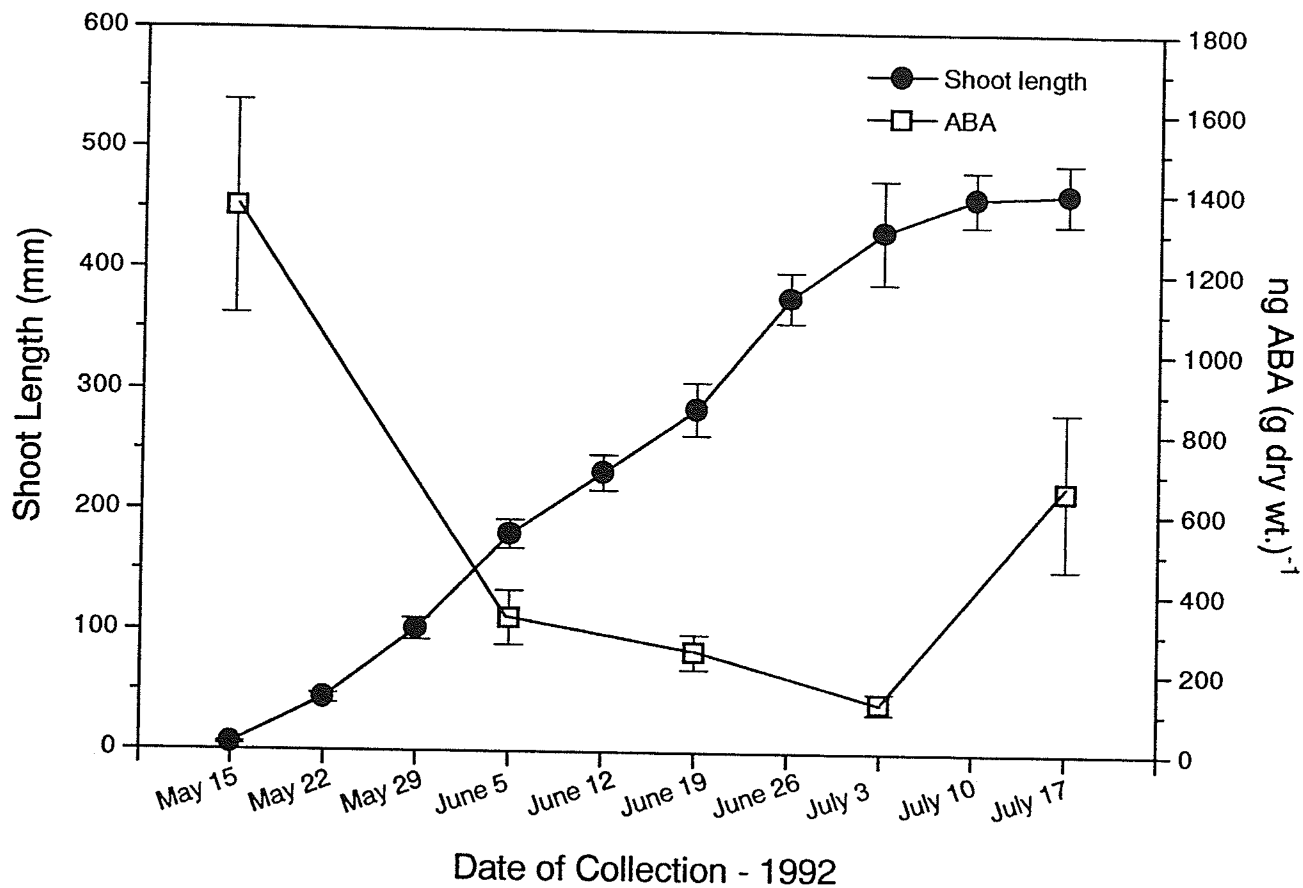


Fig. 9. Shoot expansion and corresponding ABA concentrations over the 1992 growing season. Means (± standard error) for each date were based on measurements of 10 shoot lengths and 4 ABA samples.

Leaf Production and Bud Development

Preformed foliage leaves for the current shoot were observed emerging from the bud at the first sampling date. Leaf production and expansion continued until June 26. At this time the new terminal bud for the following season was clearly visible. There was a mean of 18±0.67 foliage leaves produced on the sampled shoots. Following the production and expansion of current season foliage leaves, cataphyll or bud scale initiation for the new terminal bud occurred for approximately five weeks (Fig. 10). There was a mean of 7.0±0.83 cataphylls in the developing bud and this corresponded to the number of fully expanded cataphylls observed in the early stages of shoot expansion (7.2±0.52, n=22). It was during this stage of development that the mid season peak of ABA occurred (Fig. 10). Following the production of cataphylls, about 2 or 3 preformed foliage leaf primordia emerged. After foliage leaf initiation, the shoot apices transformed to the production of inflorescences. This period was recognized as a rapid proliferation of bracts and by September 11, floral buds were clearly visible in the bract axils. During the period of inflorescence development, ABA concentrations increased (Fig. 10).

Vegetative Maturity

In the population of orchard-grown *A. alnifolia* investigated in 1992, the last date that lateral vegetative buds burst following defoliation was July 10 (approximately 20% burst). By July 17, no buds would break and this was considered to be the time of vegetative maturity (Fig. 10). Vegetative maturity coincided with the mid season peak of ABA.

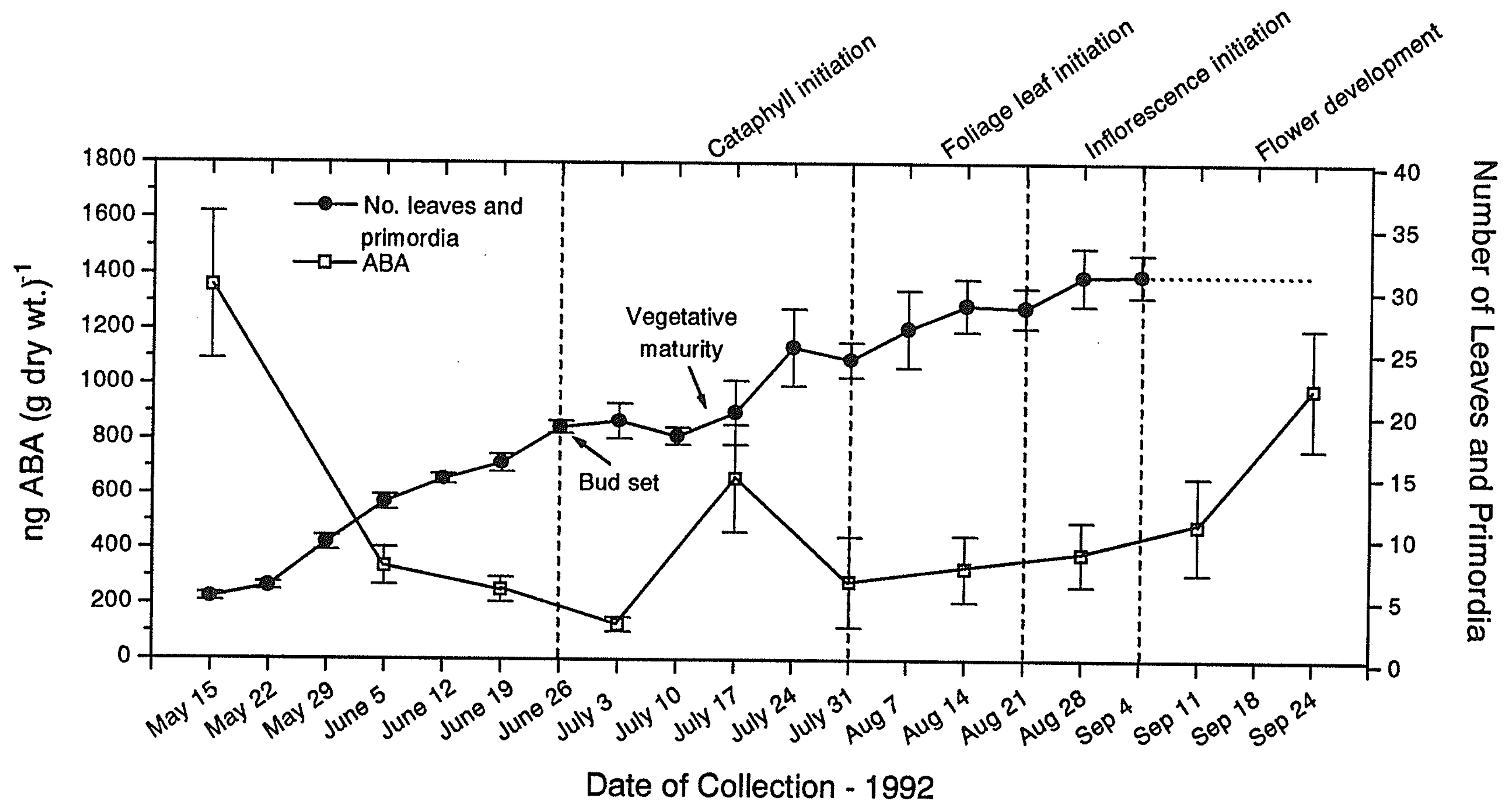


Fig. 10. Summary of leaf and primordium production patterns by the terminal shoot apex with corresponding seasonal ABA concentrations. Means (± standard error) were based on 10 shoots and 4 ABA samples at each date. Dotted line at the end of inflorescence initiation indicates that no more leaf primordia were initiated because the shoot had converted to flowering.

DISCUSSION

The overall seasonal pattern of ABA concentrations in *A. alnifolia* showed a decrease in the spring, a mid season peak followed by slight decline, and then a steady rise towards autumn. Typically, inhibitor levels are lowest at the time of bud burst (Seeley and Powell 1981; Rodriguez *et al.* 1991). It is not clear why ABA was not at its lowest concentrations in *A. alnifolia*, but there are reports where inhibitor levels are still dropping after the shoots have begun to expand (Harrison and Saunders 1975). Wright (1975) reported that ABA concentrations may increase slightly as the shoots of woody plants begin to expand due to a leaf water deficit induced in the young delicate leaves by their sudden exposure to a harsh environment. It is possible that water deficits may be involved in the response in *A. alnifolia.* However, the relationship between ABA concentration and water stress for *A. alnifolia* would need to be specifically tested in controlled experiments and concentrations monitored in the later part of the dormant period.

The ABA concentrations of *A. alnifolia* shoot tips in this study fluctuated from the highest observed value of 1354 ng ABA (g dry wt.)$^{-1}$ to a minimum of 125 ng ABA within a growing season. A range in ABA values was also observed in other species and plant parts. In sugar maple buds, Dumbroff *et al.* (1979) reported that there was an ABA concentration as low as 75 ng ABA and a high around 2000 ng within a growing season while sugar maple stems went from a similar low to a high of approximately 4000 ng ABA. Similarly, Webber *et al.* 1978 observed that in Douglas-fir, the highest concentration of bud ABA in the autumn was 2100 ng (g dry wt.)$^{-1}$ while maximum ABA

concentration of needles occurred in July at 790 ng ABA.

The declining ABA concentrations observed at the beginning of the season corresponded to the period of shoot expansion. The elongation of shoots of *A. alnifolia* continued approximately 5 weeks later than what was observed in natural populations growing in Saskatchewan (St. Pierre 1991; Steeves and Steeves 1990). The extended period of growth observed in this study was attributed to the *A. alnifolia* being young and vigorous and developing neoformed foliage leaves. Steeves and Steeves (1990) observed that shoots are ordinarily of the preformed type, although they did suggest that there are occasions where expansion of neoformed leaves may occur.

The occurrence of neoformed shoot growth resulted in a corresponding delay in cataphyll initiation. Steeves and Steeves (1990) reported a similar delay. During the time of cataphyll initiation, shoot growth stopped. Following the formation of cataphylls, preformed foliage leaf initiation occurred and then inflorescence initiation. This corresponded to a steady increase in ABA (Fig. 10) and flower development occurred as ABA rose dramatically.

The rise in ABA coinciding with inflorescence initiation and subsequent flower development may indicate that ABA is involved in the conversion. Steeves and Steeves (1990) suggested that in *A. alnifolia* endogenous factors were involved in the conversion of previously vegetative shoots to inflorescences since transformation always occurs at a defined phase of vegetative development which may vary in timing from year to year. Seidlova *et al.* (1981) were able to show that the change in direction of apical growth in *Chenopodium*, from a flat and wide vegetative apex to a more vertical floral apex, could

be manipulated by ABA and gibberellin. Seidlova (1985) summarized the effects of exogenous growth regulators on apical growth and morphogenesis in *Chenopodium*, providing some examples of possible participation of growth regulators in changing the vegetative apex into a floral one.

There was an ABA peak in the middle of the 1992 growing season which coincided with the cessation of shoot expansion. A similar relationship between cessation of elongation and a mid season peak of ABA was also reported by others (Alvim *et al.* 1976; Seeley and Powell 1981; Rodriguez and Sanchez-Tames 1986; Rodriguez *et al.* 1991). Shoot growth can be affected by environmental conditions such as water, nutrient supply, and temperature but it is clearly a complex interaction of environmental and endogenous factors that induce cessation of growth (Barros and Neill 1987). Lenton *et al.* (1972) and Hocking and Hillman (1975) failed to establish a correlation between the inhibitor, ABA, and bud dormancy. However, many other researchers stated that the growth inhibitor, ABA, is responsible for the arresting of growth and bud set (Phillips and Wareing 1958, 1959; Seeley and Powell 1981; Rodriguez *et al.* 1991). In the present study, elongation of *A. alnifolia* shoots essentially ceased at a time when ABA concentrations increased to a mid season peak (Fig. 10), suggesting that ABA may be involved in shoot growth cessation. Verification of this peak would require ABA analysis at more frequent intervals over more than one growing season.

The cessation of shoot elongation coincided approximately with the date of vegetative maturity. The time of occurrence of vegetative maturity was considerably later than previously reported for natural populations (Friesen and Stushnoff 1989). Vegetative

maturity, which prevents further growth of shoots, is thought to be an adaptation to stress (Kaurin *et al.* 1984) and improves winter survival if defoliation should occur because of drought, hailstorms, or insect infestation late in the season (Friesen and Stushnoff 1989). The apparent delay in vegetative maturity observed in the present study appears to be related to the occurrence of neoformation which extended the period of shoot growth.

Once shoot growth ceases, the buds enter a state of summer dormancy. Summer dormancy has also been called quiescence (Samish 1954) or early rest (Vegis 1964). During summer dormancy, lateral buds are dormant either because of unfavourable environmental conditions or correlative inhibition of neighbouring organs (Fuchigami *et al.* 1982). During summer dormancy leaf primordium production may still actively be continuing in the bud (Wareing 1956).

The interpretation of winter dormancy may not be as clear. As the shoots reach vegetative maturity, they pass into the state of winter dormancy where buds can no longer be stimulated to grow following defoliation. However, Wareing (1956) defined winter dormancy as the stage where growth of the bud is complete, that is, leaf primordium initiation has ceased. In the present study, primordium production in *A. alnifolia* continued for several weeks after the point where defoliation could not stimulate dormant lateral buds to break. In other words, the time of winter dormancy as defined by Wareing (1956) does not appear to correspond with that of Fuchigami *et al.* (1982). One of the explanations for this discrepancy may be related to the observation that there are phases in the development of dormancy (Vegis 1964; Fuchigami *et al.* 1982). Following the onset of winter dormancy, Fuchigami *et al.* (1982) recognized a deepening of the rest

phase beyond the point of vegetative maturity. The cessation of primordium production may in fact correspond to the stage of deepening rest.

The results of this study suggest that endogenous ABA is involved with many aspects of *A. alnifolia* shoot growth and development. Decreasing ABA concentrations correspond to increasing shoot growth while ABA accumulation mid season coincided with shoot growth cessation and vegetative maturity. Preformed primordium production continued in the bud for weeks following vegetative maturity, even though the buds themselves could no longer be stimulated to break and regrow.

CHAPTER 2

ABA concentrations in shoot tip culture and their relation to culture date and stage in culture of *Amelanchier alnifolia*

ABSTRACT

Abscisic acid (ABA) concentrations were measured in *Amelanchier alnifolia* Nutt. (saskatoon) shoot tips before culture and then at the end of the multiplication and rooting stage for each of the three culture dates over the course of one growing season. Significant differences existed in ABA concentrations of explants excised at various times in the season but by the end of the multiplication stage in culture, ABA values were similar. At the end of the rooting stage, there were differences in plantlet ABA concentrations between collection dates and more ABA was found in non-rooted plantlet than in rooted ones. Only the first culture date showed significant changes in the concentration of ABA between the time the explant was taken and the end of the multiplication stage, and then from the end of the multiplication stage to the end of the rooting stage. ABA was found to be of little value as an indicator of performance under the experimental conditions used, and there was only a weak relationship between ABA concentration and decreased plantlet quality. ABA variation among shoots and plantlets produced *in vitro* could be explained to some extent by jars used in the multiplication stage and not explants, but much of the difference could not be accounted for by the variables tested.

INTRODUCTION

Amelanchier alnifolia Nutt. (also known as saskatoon on the Canadian prairies), is a fruit bearing shrub which is becoming a more important commercial crop (Steeves and Steeves 1990). Problems have been associated with its commercialization because it is difficult to obtain both large quantities of plants (Harris 1976; St. Pierre 1992) and uniform plants that would be needed for mechanical harvesting (Casement 1976; Pruski *et al.* 1990).

Micropropagation, an asexual form of propagation under aseptic conditions *in vitro*, has the potential to produce a rapid and steady supply of genetically uniform plants. There has been considerable progress made in the micropropagation of *A. alnifolia*. Harris (1985) refined basic procedures and investigated optimal conditions needed for establishment and rapid multiplication of *A. alnifolia* shoot tips in culture. He also assessed the effect of culture conditions used in the multiplication stage on subsequent rooting. Pruski *et al.* (1990) reported on the influence of explant source, media composition on culture establishment, and BAP (6-benzylaminopurine) levels on shoot multiplication. In addition, he examined auxin effects on *ex vitro* rooting of the shoots, and the release of summer dormancy that occurs after rooting with BAP and GA_{4+7}.

Despite these advances, there are still problems associated with the micropropagation of *A. alnifolia*. One area of concern with *in vitro* production in general, according to Smith and McClelland (1991), is inconsistency in performance, quality, and survival of plantlets that result from minor procedural discrepancies *in vitro* (e.g., differences between labs in standards, schedules, techniques). One way to improve the

quality of micropropagated plants is to gain a better understanding of the physiological processes involved in the control of plant growth and development *in vitro.*

Abscisic acid (ABA) is a plant growth regulator that was originally recognized for its role in abscission and dormancy but it is now implicated in a variety of physiological processes in higher plants (Addicott and Van Steveninck 1983; Creelman 1989). ABA is suspected to be involved in the inhibition of growth *in vitro* (Barros and Neill 1987). Moreover, ABA may be responsible for post rooting dormancy since it was reported in preliminary studies to accumulate during the rooting stage *in vitro* (Pruski, personal communication). In *Hevea brasiliensis,* Haffner *et al.* (1991) reported that reactivity, that is the ability of the explant to be cultured, was affected by ABA. Increased endogenous ABA in culture reduced both the number of shoots produced and the rooting ability of the resulting plantlets.

Seasonal variation in ABA concentrations in *A. alnifolia* has been previously demonstrated (Chapter 1) and the question was raised as to whether the concentration of ABA in the shoot tip when it was excised affects the performance and quality of the explant in culture. Haffner *et al.* (1991) showed that a relation exists between the explant reactivity of *H. brasiliensis* and endogenous ABA concentrations at the time when shoot tips are excised. However, there have not been any similar studies in *A. alnifolia.*

Therefore, in view of the possible role of ABA in culture, the objectives of this paper were to: (i) determine if differences in explant ABA concentrations at the time of culture are retained during the culture process; (ii) compare ABA concentrations at various stages of the culture process, for explants taken at different collection dates; (iii)

examine the relationship of ABA concentrations to rooting and to *in vitro* plantlet morphological features (i.e. leaves and roots) (iv) quantify potential sources of variation in ABA concentrations due to culture conditions.

MATERIALS AND METHODS

Plant Material

Amelanchier alnifolia plants of the cultivar 'Smoky' located at a commercial operation located 93 km south-west of Winnipeg, Canada, were used in this experiment. Actively growing vegetative shoot tips on adventitious shoot complexes (suckers) arising from the periphery of established plants were used as a source of explants.

Shoot Tip Culture

In preparation for culture, eight vegetative shoot tips were randomly selected, trimmed to 10 mm lengths, and all opened leaves (leaves unfolded along the midrib) were removed. The shoot tips were then washed for 30 minutes in sterile deionized water with two drops of tween 80 added to 1000 ml of water (that was stirred constantly with a magnetic stirrer), rinsed in 70% ethyl alcohol for one minute, and sterilized in a Javex solution (5.25% sodium hypochlorite). The strength of the Javex solution and the length of time the shoot tips were sterilized was adjusted from a 20% solution for 30 minutes at the beginning of the growing season when contamination was a serious problem, to a 10% solution for 20 minutes for shoot tips taken at the end of the end of the season when

contamination lessened. After sterilization, the explants were washed 3 times with sterile deionized water in a Laminar Flow Bench and trimmed to a length of 6-8 mm for inoculation.

The explants bypassed the establishment stage and went directly into the multiplication stage. (Preliminary studies done the previous year on micropropagation, indicated that explants put directly into multiplication medium shorten the culture process by four weeks and produced the same number of shoots as those explants put into an establishment stage and then transferred to a multiplication stage.) The multiplication medium was MS (Murashige and Skoog 1962) and 10 μM of the hormone BAP were added. This concentration was based on the findings of Pruski (1987) who reported that the optimal concentration for shoot multiplication for several cultivars of *A. alnifolia* was between 8.88 and 13.3 μM BAP. The medium also contained 1% sucrose and was solidified with 0.6% agar. Media were sterilized in an autoclave at 121^{0}C for 20 minutes.

The explants were inoculated onto 30 ml of medium in 237 ml glass jars with plastic tops screwed on loosely. After eight weeks the explants were subcultured twice (a four week period each time) on MS medium containing 5 μM BAP to produce a minimum of six to eight shoots per explant.

Following multiplication, shoots were transferred to 125 ml glass jars (baby food jars) with screw top lids containing 15 ml of Woody Plant Medium (WPM, Lloyd and McCown 1980) for rooting. The shoots were initially placed onto WPM with 10 μM of IBA (indole-3-butyric acid) for six days and then WPM without any hormone for the rest of the four-week rooting period.

Cultures vessels were arranged on a stand in rows that were parallel with the light fixtures but well back from the shelf edges. Fluorescent light was used at a mean irradiance of 52 $\mu E \cdot m^{-2} \cdot s^{-1}$ for a 16 hour photoperiod and room temperatures were maintained at 23 ± 2° C.

Experimental Design

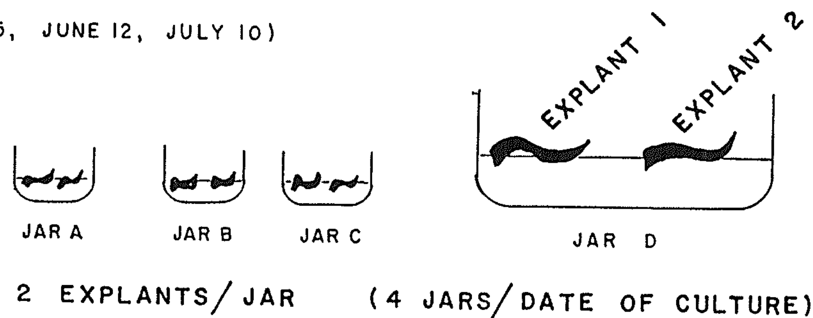
For this experiment, cultures were set up at the beginning, middle, and end of the active growth period at four-week intervals (Fig. 11). At each "date" (d), eight shoot tips were randomly selected and used as explants. Shoot tips were excised in the field and transported back to the laboratory in a cooler.

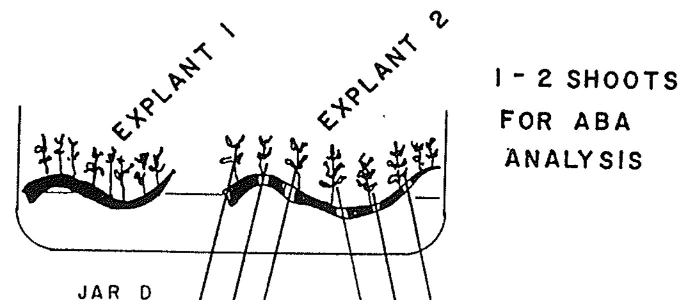
The experimental design was that of a nested ANOVA. Initially there were two "explants" (e) inoculated into a "jar" (j) containing multiplication medium. There were four such jars per "date" (d) (Fig. 11). Following multiplication, six shoots from each explant were transferred into "new jars" (n) for rooting. This was accomplished by placing three shoots in each of two new jars used for rooting. The result was a total of 16 jars per culture date.

Analysis of Plantlets

Morphological Measurements

Measurements were made at the end of the rooting stage for each shoot, now considered a plantlet. The total number of leaves and the largest measured leaf length were recorded. It was noted whether the plantlet had rooted or not. If it had, the number





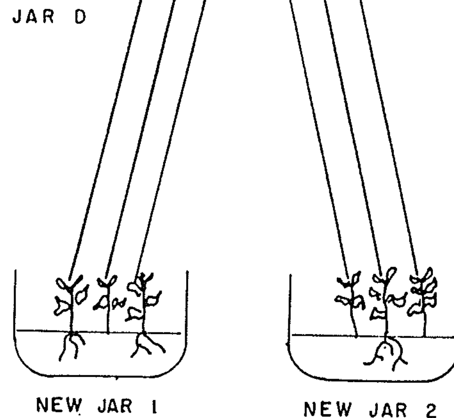


Fig. 11. Schematic diagram of the experimental design. Two explants were inoculated into each of four jars at the beginning, middle, and end of active growth. The shoots produced by the explant at the end of the multiplication stage were then followed through the rooting stage or used for ABA analysis.

of roots was counted and the length of the longest root was recorded. Each plantlet was rated according to its overall appearance and vigour. A rating of 5 was excellent and reflected a plantlet that had many good sized, healthy looking leaves of medium green. A plantlet with a rating of 1 was very poor with fragile, short stems and few, narrow, poorly developed leaves. To minimize stress on the plantlets, these measurements were made quickly and then the plantlets were frozen and stored in a -70° C freezer for ABA analysis.

ABA Analysis

ABA concentrations were analyzed at the three major points in the culture process for each of the culture dates (Fig. 11). The first ABA reading was made at the time when explants were excised from the field. By necessity, shoot tips used for ABA analysis were separate from those used as explants because of the destructive nature of the test used to determine ABA values. The second ABA reading was obtained at the end of the multiplication stage from the best of the shoots that remained following the transfer of six shoots per explant to the rooting stage (Fig. 11). (There was a variation in the quality of these surplus shoots since some explants produced fewer good quality shoots than others.) The third reading was obtained at the end of the rooting stage by evaluating the ABA of each plantlet.

Prior to ABA analysis, all samples were stored in a -70° C freezer. Processing involved freeze-drying each sample separately and then grinding it into a powder. The dry weight used for ABA analysis was approximately 0.01 g for the *in vitro* samples and

the May 15 *in vivo* samples. The June 12 and July 10 *in vivo* samples were 0.1 g. Methanol extraction and resuspension of each sample followed by the indirect ELISA test to analyze ABA concentrations were done according to the protocol established by Walker-Simmons (1987). For a more detailed description of the procedures adapted for *A. alnifolia*, see Chapter 1.

Statistical Analysis

A variety of statistical tests were performed using Statistical Analysis Systems (SAS User's Guide 1985). One way analysis of variance (ANOVA) was used to compare the ABA concentrations between culture dates. This test determined whether the field samples at one culture date had significantly more ABA than another.

Student's t-test was used to assess ABA differences between field samples and values taken at the end of the multiplication stage. A paired t-test was used to test differences between the multiplication stage and the rooting stage for each of the three culture dates. A t-test also compared ABA concentrations between rooted and non rooted plantlets. The t-test was used since samples were unrelated and independence of sampling could be assumed (Milliken and Johnson 1984).

At the end of the multiplication stage, the following model was used to compare ABA concentrations (Y) in relation to the overall mean (μ) of dates (d), jars within dates j(d), and explants within jars within dates e(dj):

[1] $$Y = \mu + d + j(d) + e(dj)$$

At the end of the rooting stage, new jars within explants within jars within dates n(dje) was added to the model. Samples tested were not independent because the shoots used at the end of the multiplication stage and the plantlets at the end of the rooting stage could be traced back to the original explant. As a result these samples were related and this correlation was taken into account when making the comparison (Milliken and Johnson 1984).

A method-of-moments estimation (Milliken and Johnson 1984) was performed to quantify potential sources of variation due to jars, explants, and new jars. Method-of-moments is an estimation technique which generates ratios to determine the percentage of each component relative to the total variance.

Correlation analysis was used to assess associations between factors such as leaf number, largest leaf length, root number, root length, vigour ratings, and ABA.

RESULTS

Shoot Production at the End of the Multiplication Stage

There was variation in both the number and quality of shoots produced per explant at the end of the multiplication stage among culture dates. The mean number of shoots (± standard error) for the May 15 culture was 11.9±3.7, which was similar to the July 10 mean of 11.8±5.2. However, the last culture date of July 10 showed a greater variation in the number of shoots that were produced per explant. The mid culture date at June 12 produced fewer shoots (8.3±1.2) than the other two culture dates but was more consistent in the number of shoots per explant. Some of the shoots were of poor quality with short fragile stems and narrow, under developed leaves.

Relationship of ABA Concentrations to Morphological Features

At the end of the rooting stage, the general relationship between quantitative morphological features and ABA concentrations was investigated by taking the data from each plantlet and pooling the results for correlation analysis. In general, there was a moderate negative correlation between ABA and plantlet morphology. Of the plantlet features tested, ABA concentration showed a slightly higher correlation with leaf length ($r=-0.35$), number of roots ($r=-0.34$), and vigour rating ($r=-0.34$) than root length ($r=-0.30$) or number of leaves ($r=-0.28$) (Table 1). These results suggest that as ABA increases, there is a tendency for the number and length of leaves and roots produced by the plantlet to decrease.

Table 1. Correlation matrix relating mean ABA concentration (ABA), vigour/appearance rating (Rating), number of leaves, number of roots, length of root (mm), and length of leaf (mm). r is the Pearson correlation coefficient and p is the probability of a larger r.

	Rating		Number of Leaves		Number of Roots		Root Length		Leaf Length	
	r	p	r	p	r	p	r	p	r	p
ABA	-0.336	0.0001	-0.280	0.0016	-0.344	0.0001	-0.303	0.0006	-0.348	0.0001
Rating			0.547	0.0001	0.505	0.0001	0.435	0.0001	0.611	0.0001
Number of Leaves					0.241	0.0066	0.181	0.0427	0.433	0.0001
Number of Roots							0.442	0.0001	0.395	0.0001
Root Length									0.295	0.0008

The vigour ratings, an indicator of quality, were positively correlated with an increase in size and number of organs - notably leaf number (r=0.55), and leaf length (r=0.61) (Table 1). There was also a correlation between rating and root number (r=0.51) and root length (r=0.44). In general, the plantlets that had a rating of 5 and 4 rooted more frequently than those with a rating of 3 or less (Table 2).

Table 2. Relationship between rating and the percentage of rooted plantlets.

Rating	Number of Plantlets	% Rooted
5	31	84
4	36	64
3	46	20
2	11	9
1	3	0

Shoot Quality and ABA Concentrations Among Dates and Culture Stages

There were no significant differences between date at the end of the rooting stage and the morphology of the plantlets produced at the end of the rooting stage (rating, p=0.90; number of leaves, p=0.22; number of roots, p=0.42; root length, p=0.37; leaf length, p=0.12). This meant that morphological features such as number of leaves and roots, length of leaves and roots, and an overall vigour rating of plantlets produced at the end of the experiment were similar regardless of the culture date they originated from.

The concentration of ABA was significantly different between explants taken for culture at the beginning, mid and end of the growing season (p=0.0008). Explant ABA

concentration on May 15 was high (1731±153 (± standard error) ng per g dry weight), and then it began to drop so that by July 10, the concentration of ABA in explants was 717±140 ng (Fig. 12).

At the end of the multiplication stage, the concentration of ABA was not significantly different between dates ($p=0.51$). ABA concentrations in tissue sampled on May 15 dropped significantly from the time the explants were taken to the end of the multiplication stage ($p=0.0007$), followed by a similar but non significant trend for June 12. There was relatively no change in ABA for July 10 (Fig. 12).

In contrast to the multiplication stage, ABA concentrations were again significantly different between dates ($p=0.02$) by the end of the rooting stage. This was accounted for by a significant ABA increase which occurred between the end of the multiplication stage to the end of the rooting stage on May 15 ($p=0.001$, Fig. 12).

At the end of the rooting stage, ABA analysis revealed that non rooted plantlets had more ABA than those that rooted (Fig. 12). The difference in ABA concentrations between rooted and non rooted plantlets was significant for May 15 and June 12 but not for July 10 (Fig. 12). The percentage of rooted plants was 44% for May 15, 45% for June 12, and 51% for July 10.

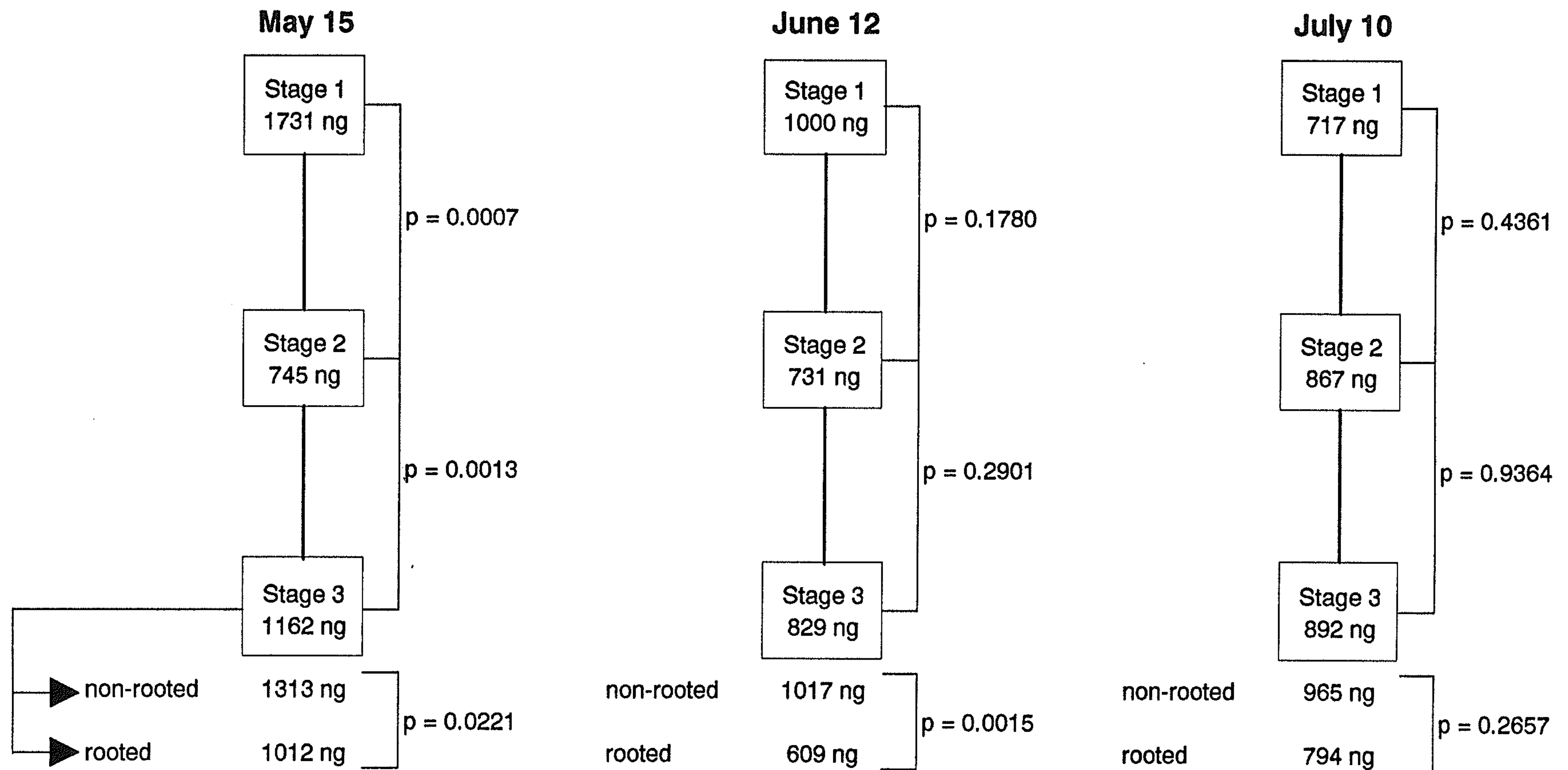


Fig. 12. The relationship of mean ng ABA (g dry weight)$^{-1}$ to stage of culture for each of the three dates in the 1992 growing season. Stage I is when the shoot tip was excised. Stage II is at the end of the multiplication stage. Stage III is at the end of rooting stage. For Stage III, the sample was divided into rooted and non-rooted plantlets. The p values are derived from t-tests.

Variation in Morphological Traits and ABA in Relation to Culture Vessel and Explant Source

Culture Vessel

At the end of the rooting stage, plantlet morphology and ABA concentrations were compared in relation to the vessels used both for the multiplication of shoots and subsequent rooting. At the end of the experiment, it was observed that there was no significant difference among the 45 culture vessels used for rooting in relation to the quantitative expression of any of the morphological traits examined (rating p=0.41, leaf number p=0.14, root number p=0.20, leaf length p=0.83, root length p=0.54).

The morphology of plantlets in relation to the jar used in the multiplication stage was also compared at the end of the experiment. There were significant differences in ratings (p=0.01), leaf number (p=0.07), and root number (p=0.03) (Table 3). The lengths of the longest roots and leaves were not significantly different.

ABA concentrations were compared among culture vessels at each stage of culture. At the end of the experiment, comparisons of plantlets produced in the same rooting jars revealed no significant difference in the ABA concentration (p=0.57). Moreover, no variation in ABA concentrations was accounted for by these original vessels according to the methods-of-moments estimation.

Table 3. Mean (± s.e.) rating, leaf and root number, leaf and root length (mm) per jar for the three dates in 1992. p is the probability of a larger F-statistic in the nested ANOVA.

Parameter	May 15	June 12	July 10	p Value
Jar A				
rating	4.2±0.27	3.9±0.27	4.0±0.27	
leaf number	5.7±0.48	4.4±0.48	4.8±0.48	
root number	0.67±0.41	0.92±0.41	1.7±0.44	
root length	26.0±7.0	16.7±7.0	27.2±7.5	
leaf length	17.3±1.5	18.2±1.5	14.4±1.5	
Jar B				
rating	3.7±0.28	4.1±0.27	3.3±0.30	
leaf number	4.8±0.51	4.8±0.48	4.7±0.54	
root number	0.54±0.44	0.58±0.41	0.42±0.46	
root length	12.5±7.5	14.2±7.0	12.7±7.9	
leaf length	18.3±1.5	18.5±1.5	16.3±1.6	
Jar C				
rating	3.7±0.27	4.0±0.27	3.7±0.35	
leaf number	5.9±0.48	6.1±0.48	4.5±0.64	
root number	0.83±0.41	2.3±0.41	1.3±0.54	
root length	23.1±7.0	15.9±7.0	13.8±9.3	
leaf length	16.8	20.6±1.5	15.9±1.9	
Jar D				
rating	3.5±0.30	2.3±0.28	3.2±0.38	
leaf number	5.4±0.54	3.7±0.51	4.7±0.68	
root number	0.50±0.46	0.96±0.44	0.33±0.58	
root length	23.8±7.9	17.9±7.5	13.3±9.9	
leaf length	16.6±1.6	16.1±1.5	17.5±2.1	
Mean				
rating	3.7±0.14	3.6±0.14	3.6±0.16	0.01
leaf number	5.5±0.25	4.7±0.24	4.6±0.29	0.07
root number	0.64±0.21	1.2±0.21	0.94±0.25	0.03
root length	21.3±3.7	16.2±3.6	16.7±4.3	0.92
leaf length	17.3±0.76	18.3±0.74	16.0±0.90	0.81

When ABA concentrations of plantlets at the end of the rooting stage were compared in relation to the jars that were used for their multiplication, the means for each jar appeared to vary (Fig. 13). However, the standard errors were large and the analysis revealed no significant difference among jars (p=0.39). Differences attributable to jars at the multiplication stage accounted for only 1.8% of the variation in ABA.

ABA concentrations were also compared at the end of the multiplication stage, that is analysis of shoots not transferred to a rooting medium. This analysis revealed no significant differences among jars within dates (p=0.11). In contrast to the analysis at the rooting stage, variation within jars was relatively low, except for the June 12 date (Fig. 14). In fact, method-of-moments estimation revealed that differences among jars accounted for 52% of the variation in ABA.

Explant Source

At the end of the experiment (rooting stage), there were no significant differences in either the quantitative expression of morphological traits, except for leaf length (p=0.08), or ABA concentrations (p=0.38) among plantlets derived from different *A. alnifolia* explants. Only a small amount of variation in ABA was accounted for by explant differences (2.2%).

At the end of the multiplication stage, ABA concentrations for shoots were not significantly different among explant sources (p=0.92). At this stage, the difference among explants did not account for any variation in ABA.

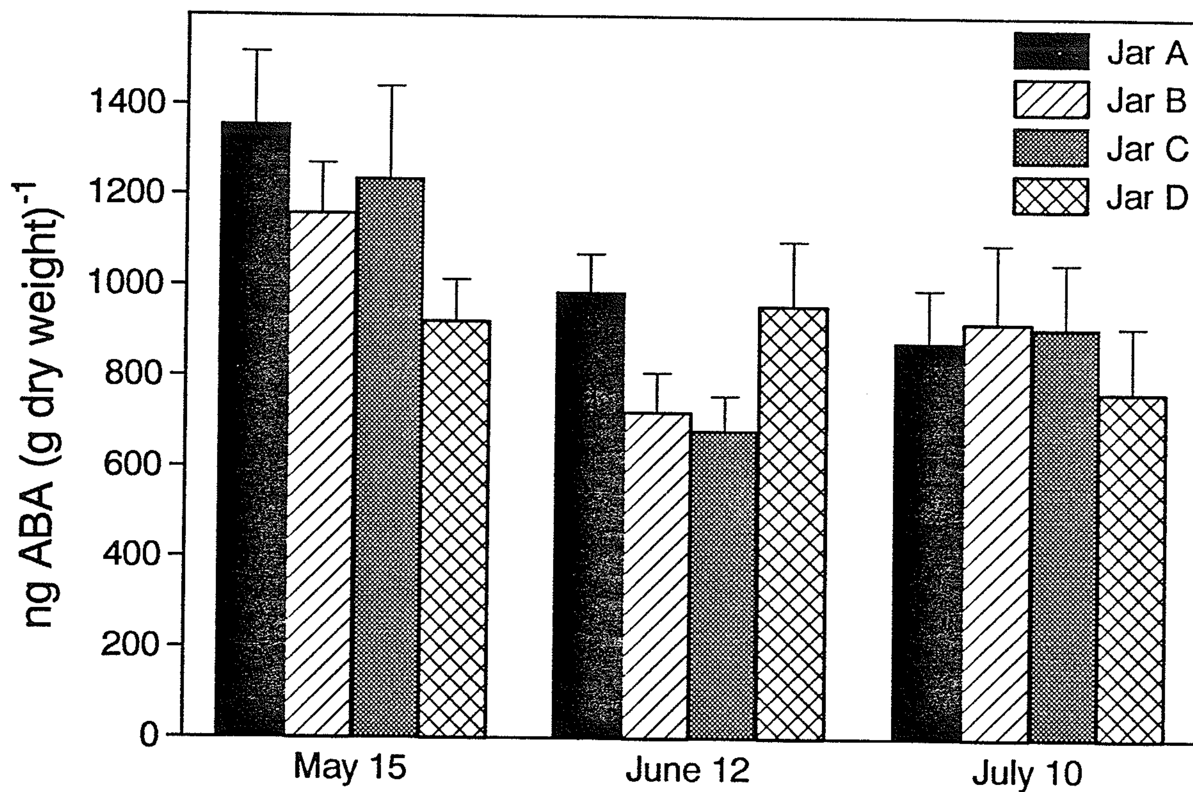


Fig. 13. Mean ng ABA (g dry weight)$^{-1}$ (± standard error) in 12 plantlets that originated in the same jar in the multiplication stage and were analyzed at the end of the rooting stage for each of the three dates in 1992.

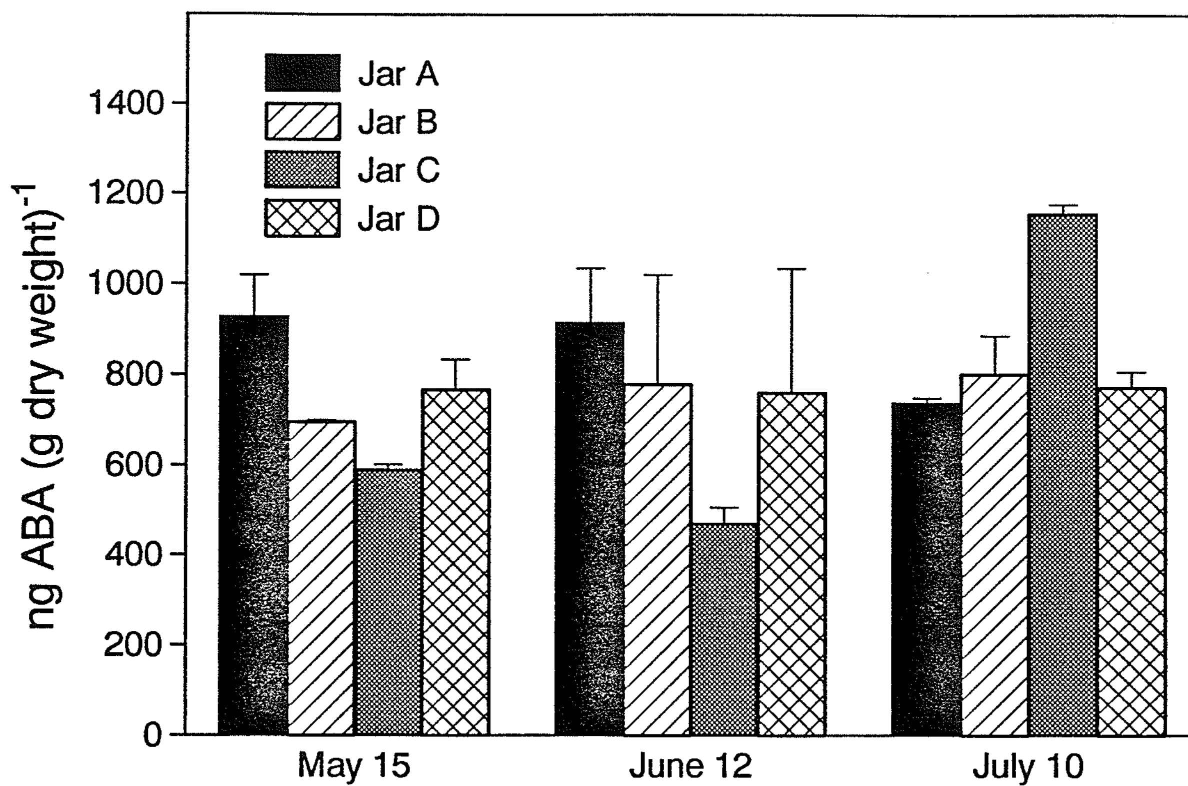


Fig. 14. Mean ng ABA (g dry weight)$^{-1}$ (± standard error) at the end of the multiplication stage for shoots in each jar from each culture date in 1992.

As a result of the small amount of variation in ABA explained by both the explant source material and culture vessels tested at each stage of culture, there was a large component of variation that was not accounted for by the conditions tested (48% at the end of the multiplication stage and 96% at the end of the rooting stage).

DISCUSSION

The quality of a shoot produced *in vitro* can be assessed by quantitative morphological features such as shoot length, and area (Smith and McClelland 1991). A relationship between quality of a plantlet and endogenous ABA in the explant at the time it was taken has been shown by Haffner *et al.* (1991) for *Hevea brasiliensis* in which increased ABA was associated with reduced numbers of shoots and roots. In *A. alnifolia*, there was only a moderate negative correlation between ABA concentrations and quantitative morphological traits like leaf and root number, leaf and root length and suggests that the two are not closely associated. However, a direct relation was reported to exist between shoot quality and rooting capacity of *Amelanchier* (Harris 1985; Smith and McClelland 1991) and was similarly demonstrated in *A. alnifolia* with those plantlets that had a rating of 5 and 4 rooting more frequently than those that had a rating of 3 or less. McClelland and Smith (1990) suggest that increased rooting capacity is due to greater leaf area, since rooting cofactors are produced in the leaves and control, in part, the rooting process.

The suggestion that ABA and plantlet performance are not necessarily closely associated comes from a comparison of ABA in relation to date both when the explants were coming into culture and in the rooting stage, with the quantitative morphological traits of plantlets at the end of the experiment. No significant differences in quantitative morphological features of plantlets in relation to date were observed, even though there were significant differences in ABA concentrations among date both at the beginning and again at the end of the experiment.

Differences in ABA concentrations that existed in explants coming into culture at various times in the growing season (Chapter 1), were not present at the end of the multiplication stage when shoots produced by the explants were analyzed. Noiton *et al.* (1992) observed pronounced ABA differences between greenhouse plants and cultured shoots and attributed the lower ABA in cultured shoots to a reduction of water stress. Similarly, the only date where there was a significant decrease in *A. alnifolia ABA* concentrations from the time the explant was taken to the end of the multiplication stage occurred on the first date when initial ABA concentrations may have been high due to an induced water deficit caused by to the sudden exposure of delicate young leaves into a harsh environment (Chapter 1).

Similarities in ABA concentrations at the end of the multiplication stage may be due in part to the culturing process itself. The culture of shoot tips in this study involved the removal of their leaves before inoculation and according to Kozai (1991), shoots and leaves produced *in vitro* are not functionally the same as those growing *in vivo*, particularly when the culture conditions include sugar in the medium, low light, and a low

CO_2 concentration in the vessel. Similarly, Bouza *et al.* (1994) observed that *Paeonia suffruticosa* explants lost their capacity to regulate hormone metabolism in culture. Thus *A. alnifolia* explants taken at different collection dates may have produced similar endogenous ABA concentrations at the end of the multiplication stage because of heterotrophic growth caused by culture conditions.

At the end of the rooting stage, plantlets from the first culture date showed a significant rise in ABA whereas in the last two culture dates no such increase was observed. The results from a preliminary study by Pruski (personal communication) showed that during the rooting process the concentration of ABA in plantlets increased with time and that the highest ABA concentration occurred at the end of the rooting period. In addition, he suggested that these high ABA concentrations may explain why most *A. alnifolia* plantlets are dormant at the end of the rooting period. However, because of variation among culture dates in relation to accumulation of ABA in the rooting stage, a trend in ABA concentrations could not be conclusively demonstrated in this present study. Further testing, probably with a larger number of samples, would be necessary to validate the existence of patterns.

ABA can be involved in the regulation of root formation with either stimulatory or inhibitory effects, depending on the species treated and the amount of hormone applied (Maldiney *et al.* 1986). In *in vitro* studies done by Maldiney *et al.* 1986, ABA concentrations were observed to be high in tissue where the first cytological steps of the rhizogenesis would occur and then following root growth, ABA concentrations decreased considerably. They also suggest that high ABA concentrations are concomitant with a

high indole acetic acid (IAA) / zeatin plus zeatin riboside (Z + ZR) ratio. In the present study, lower concentrations of ABA were observed in rooted plantlets and higher concentrations in non-rooted plantlets, however, what is not known is the involvement and concentrations of the other hormones. Those plantlets that did not root could possibly have had a greater amount of Z + ZR which had an inhibitory effect on rooting. More research is still required before the precise mechanism of ABA action and its interaction with other root growth factors is fully understood (Addicott and Van Steveninck 1983).

The performance of plantlets, in terms of quantitative morphology and ABA concentrations, was investigated in relation to a set of same-sized vessels used for the multiplication stage and to a set of smaller sized vessels used for the rooting stage. There were no differences in plantlet morphology or ABA concentrations in relation to the vessels used for rooting. On the other hand, there were differences in morphology of plantlets at the end of the rooting stage related to jars used for multiplication. There was also variation in ABA concentrations in relation to the jars at the multiplication stage but the variation did not appear to persist into the rooting stage. Because ABA concentrations were shown to vary among jars at the multiplication stage, there is the possibility that these ABA concentrations may have persisted to affect morphological features at the end of rooting. The accumulation of hormones such as BAP (Pruski 1987) has been shown to result in shoot deformities after several subcultures. However, there was no clear relationship between the quantitative morphological features and the concentrations of ABA in relation to individual vessels at the multiplication stage (Fig. 13 and Table 3). In addition, when comparing ABA concentrations originating from specific multiplication

vessels, there was not a clear correspondence between relative ABA concentrations at the end of the experiment and concentrations at the multiplication stage (Figs. 13 and 14).

Variation of plantlet morphology in relation to vessels used in the multiplication stage may be a result of the experimental design or of slight differences in the physical microenvironment of the different vessels. Changes in the physical microenvironment including light, humidity, temperature, gaseous environment, physical growing area, were suggested by Smith and McClelland (1991) to have the potential to affect the quality of microcuttings. In this study all the vessels used for multiplication were the same size with the same ratio of explant to medium, however, what differed was the position of the vessels on the light stand in relation to the light source. Vessels near the centre of the fluorescence tube would receive a slightly higher irradiance level than those near the end of the tube. In addition, there may be slight changes in temperature depending on whether the vessel was on the edge or in the middle of the light stand. Possibly jar closure may have varied slightly resulting in variation in gaseous exchange rates as well as levels of ethylene and humidity (Debergh and Maene 1981).

The results of this study indicated that very little variation in either ABA in shoots at the end of the multiplication stage or ABA and morphological features of plantlets at the end of the rooting stage could be explained by initial explant. However, there was variation in the number of shoots produced by an explant at the end of the multiplication stage both within and between collection dates. The reason is unclear, although shoot production may have been influenced by other endogenous plant growth regulators in the explant such as cytokinin, gibberellin, or auxin. Plant grow promoters fluctuate

throughout the season (Seeley 1990) and it has been reported by Luckwill (1970) that concentrations of cytokinins and auxins are highest during apple tree bloom, after which there is a gradual decline until very low levels are reached at about the time shoot growth stops. In this study, some shoots appeared to have short, fragile stems with narrow, poorly developed leaves, which is similar to the description of shoots that were observed when explants were cultured in medium containing BAP in concentrations of 17.7 μM or greater (Pruski 1987). Endogenous concentrations of cytokinin together with the BAP added to the medium may have had an influence of shoot number and quality, however, endogenous concentrations of BAP in explants cultured at various time in the growing season would need to measured before the effect of BAP on shoot efficiency could be verified.

SUMMARY AND CONCLUSIONS

ABA concentrations in *A. alnifolia* were shown to fluctuate throughout the season. In the first study changes in ABA were associated with shoot growth and development. It was observed that as ABA concentrations decreased, shoot elongation increased and when ABA accumulated toward mid season, shoot elongation ceased and vegetative maturity occurred. Following vegetative maturity, ABA concentrations rose and primordium production continued in the bud even though the bud itself could not be stimulated to break and regrow. In the second study there were significant differences in ABA concentrations between the dates tested both for explants coming into culture and again for plantlets in the rooting stage. However, in contrast to the association of ABA

to shoot growth and development in field plants, *in vitro* plantlets showed only a weak correlation between ABA concentrations and plantlet quality in terms of parameters such as rating, number and length of leaves and roots. Thus the use of ABA in plantlets as an indicator of *in vitro* performance appears to be limited, at least, under the experimental conditions tested.

In addition, ABA concentrations at the end of the multiplication stage were similar despite any differences in original explant ABA which suggests that differences in explant ABA coming into culture do not appear to persist once in culture and any variation there may be in ABA is not closely correlated with quantitative plantlet morphology. Nevertheless, the concentrations of ABA in culture needs to be investigated further and future work could include sampling ABA at the beginning, mid and end of each stage of culture instead of just the end of a stage to determine a more accurate profile.

The vessels used for culture were tested in relation to ABA concentrations and quantitative morphological features of plantlets. There were differences in plantlet quality in relation to the 12 vessels used in multiplication stage but not for the vessels used in rooting. Also, differences among culture vessels at the end of the multiplication stage accounted for a considerable amount of the variation in ABA content, but by the end of the rooting stage variation in ABA in relation to the original vessel was slight. A clear relationship between quantitative expression of morphological features at the end of the rooting stage and variation in ABA concentrations at the end of the multiplication stage was not apparent, which again suggests that ABA is a weak performance indicator.

The growth and development of a plant is the result of an interaction of both growth promoters and inhibitors. ABA may reveal part of the story and the results of this study do suggest that ABA plays a role both in shoot and bud development of orchard-grown *A. alnifolia* and to a limited extent, in the performance of cultured plantlets. However, it could be important to investigate ABA concentrations in context of other plant growth regulators. Future work could include monitoring endogenous levels of IAA together with ABA, particularly in the rooting stage since *A. alnifolia* has been known to be a 'shy' rooter. Therefore research could include comparisons of endogenous concentrations of IAA and the IAA/ABA ratio on those plantlets that rooted with those that did not. In addition, the increase of exogenous concentrations of IAA could be investigated on the effect of the endogenous IAA/ABA ratio and the capacity to root.

Future research is needed to validate the seasonal pattern of ABA concentrations in orchard-grown shoot tips of *A. alnifolia*. In the first study, the seasonal trend of ABA showed a mid season peak on July 17, however, in the second study there was a suggestion of a second peak on June 12. The points used for the June 12 and July 10 culture dates in the second study were taken on weeks not used for sampling the seasonal trend, and although the ABA concentration for the July 10 culture date reinforced the seasonal trend, the ABA concentration for the June 12 culture date did not. Therefore, future research could involve weekly analysis of ABA concentrations instead of biweekly analysis in order to establish the pattern more accurately. In addition, it would be helpful to measure the ABA seasonal trend for more than one season since the pattern may vary from year to year depending on the environmental conditions. ABA is known to increase

in response to water stress and should the summer be hot and dry, the ABA seasonal pattern could be different than a summer that was cool and wet. In the summer of 1992 there appeared to be some association between increased ABA following a week of decreased precipitation (Appendix B) or increase temperature (Appendix C). However, the exact relation of ABA concentrations to drying soil conditions and the root to shoot communication with a corresponding increase in ABA concentrations in the leaves would have to be tested in controlled experiments.

In closing, this study has extended the knowledge base for *A. alnifolia* through the investigation of ABA concentrations in vegetative shoot growth of orchard-grown plants and in the production of *in vitro* grown plantlets. The results and ideas presented in this thesis may provide a basis or a direction for future work.

REFERENCES

Addicott, F.T., and Carns, H.R. 1983. History and Introduction. In Abscisic Acid. Edited by Fredrick T. Addicott. Praeger, New York. pp. 1-21.

Addicott, F.T., and Van Steveninck, R.F.M. 1983. Summary: Significance of abscisic acid in the life of plants. In Abscisic Acid. Edited by Fredrick T. Addicott. Praeger, New York. pp. 581-586.

Alvim, R., Hewett, E.W., and Saunder, P.F. 1976. Seasonal variation in the hormone content of willow. 1. changes in abscisic acid content and cytokinin activity in the xylem sap. Plant Physiol. 57:474-476.

Barros, R.S., and Neill, S.J. 1987. Shoot growth in willow (*Salix viminalis*) in relation to abscisic acid, plant water status and photoperiod. Physiol. Plant. 70:708-712.

Bell, A.D. 1991. Plant form: an illustrated guide to flowering plant morphology. Oxford University Press, New York.

Bishop, B.H., and Nelson, S.H. 1976. Progress report on the propagation and transplanting of saskatoon (*Amelanchier alnifolia*). WCSH. Reports of the proceedings of the 32nd annual meeting. pp. 68-72.

Bishop, B.H., and Nelson, S.H. 1980. Propagation and transplanting of saskatoon (*Amelanchier alnifolia* Nutt.) softwood cuttings. Can. J. Plant Sci. 60:883-890.

Bornman, C.H. 1983. Morphological, anatomical and ultrastructural responses to abscisic acid. In Abscisic Acid. Edited by Frederick T. Addicott. Praeger, New York. pp. 523-551.

Bouza, L., Jacques, M., Sotta, B., and Miginiac, E. 1994. The reactivation of tree peony (*Paeonia suffruticosa* Andr.) vitroplants by chilling is correlated with modifications of abscisic acid, auxin and cytokinin levels. Plant Sci. 93:153-160.

Casement, E.B. 1976. Outline of saskatoon rooting trials. WCSH. Reports of proceedings of the 32nd annual meeting. pp. 73-74.

Cornforth, J.W., Milborrow, B.V., Ryback, G., and Wareing, P.F. 1965. Chemistry and physiology of 'dormins' in sycamore: Identity of sycamore 'dormins' with abscisin II. Nature 205:1269-1270.

Creelman, R.A. 1989. Abscisic acid physiology and biosynthesis in higher plants. Physiol. Plant. 75:131-136.

Cumming, W.A. 1976. Evaluation and propagation of saskatoons. WCSH. Reports of proceedings of the 32nd annual meeting. pp. 76-80.

Debergh, P.C., and Maene, L.J. 1981. A scheme for commercial propagation of ornamental plants by tissue culture. Scientia Hort. 14:335-341.

Dumbroff, E.B., Cohen, D.B., and Webb, D.P. 1979. Seasonal levels of abscisic acid in buds and stems of *Acer saccharum*. Physio. Plant. 45:211-214.

Eagles, C.F., and Wareing, P.F. 1963. Dormancy regulators in woody plants: experimental induction of dormancy in *Betula pubescens*. Nature 199:874-875.

Eagles, C.F., and Wareing, P.F. 1964. The role of growth substances in the regulation of bud dormancy. Physiol. Plant. 17:697-709.

El-Antably, H. M. M., Wareing, P. F., and Hillman, J. 1967. Some responses to d,l-abscisin (dormin). Planta 73:74-79.

Friesen, L.J. 1986. Phenological and physiological development of *Amelanchier alnifolia* Nutt. during summer growth, dormancy and cold acclimation. M.Sc. thesis, Dept. Hort. Sci. U. of Saskatchewan, Saskatoon, Canada.

Friesen, L.J., and Stushnoff, C. 1989. Vegetative maturity of *Amelanchier alnifolia* Nutt. compared to red-osier dogwood and rescue crabapple. Can. J. Plant Sci. 69:955-960.

Fuchigami, L.H., Weiser, C.J., Kobayashi, K., Timmis, R., and Gusta, L.V. 1982. A degree growth stage (^{o} GS) model and cold acclimation in temperate woody plants. In Plant cold hardiness and freezing stress. Edited by P. H. Li and A. Sakai. Academic Press, New York, N. Y. pp. 93-116.

Gamborg, O.L., Miller, R.A., and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell. Res. 50:151-158.

Grainger, G.D. 1980. Propagation and growing of saskatoon. Proceedings of the C.S.H.S. 25th Annual Meeting. 19:34.

Griffiths, L.L. 1984. Some studies of propagation and culture of *Amelanchier alnifolia* (Nutt.) Nutt. M. Sc. thesis, Dept. of Plant Sc., U. of Manitoba, Winnipeg, Canada.

Haffner, V., Maldiney, R., and Lardet, L. 1991. Reactivity of *in vitro Hevea brasiliensis* (Mull. Arg.) apices: relation with their endogenous level in ausin, abscisic acid and cytokinin. C. R. Acad. Sci. Paris, 312, Series III, p.171-176.

Harris, R.E. 1976. Saskatoons (*Amelanchier alnifolia*) summary report. WCSH. Reports of proceedings of the 32nd annual meeting. pp. 50-59.

Harris, R.E. 1985. *In vitro* propagation of saskatoons. WCSH. Reports of proceedings of the 41st annual meeting. pp. 71-72.

Harris, R.E. 1989. The Saskatoon. In Saskatoon Production Manual. Prairie Fruit Growers Association.

Harris, M.J., and Outlaw, W.H., Jr. 1990. Histochemical technique: a low-volume, enzyme-amplified immunoassay with sub-fmol sensitivity. Application to measurement of abscisic acid in stomatal guard cells. Physiol. Plant. 78:495-500.

Harrison, M.A., and Saunders, P.F. 1975. The abscisic acid content of dormant birch buds. Planta 123:291-298.

Hartmann, H.T., and Kester, D.E. 1983. Plant propagation - principles and practices. 4th edition. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Henkes, R., and Dietz, J. 1992. The other berries. The Furrow, Prairie Edition / May-June.

Hetherington, A.M., and Quatrano, R. 1991. Tansley Review No. 31 Mechanisms of action of abscisic acid at the cellular level. Phytol. 119:9-32.

Hocking, T.J. and Hillman, J.R. 1975. Studies on the role of abscisic acid in the initiation of bud dormancy in *Alnus glutinosa* and *Betula pubescens*. Planta 125:235-242.

Jones, H.G., and Jones, M.B. 1989. Introduction: some terminology and common mechanisms. In: Plants Under Stress. Edited by H.G. Jones, T.J. Flowers, and M.B. Jones. Cambridge University press. Cambridge. pp 1-11.

Juntilla, O., Stushnoff, C., and Gusta, L.V. 1983. Dehardening in flower buds of saskatoon-berry, *Amelanchier alnifolia*, in relation to temperature, moisture content, and spring bud development. Can. J. Bot. 61:164-170.

Kaurin, A., Stushnoff, C., and Juntilla, O. 1984. Cold acclimation and dormancy of *Amelanchier alnifolia*. J. Amer. Soc. Hort. Sci. 109:160-163.

Kozai, T. 1991. Photoautotrophic micropropagation. *In Vitro* Cell. Dev. Biol. 27:47-51.

Kyte, L. 1983. Plants from test tubes - an introduction to micropropagation. Timber Press. Portland Oregon.

Lenton, J.R., Perry, V.M., and Saunders, P.F. 1972. Endogenous abscisic acid in relation to photoperiodically induced bud dormancy. Planta 106:13-22.

Levitt, J. 1980. Responses of plants to environmental stresses - water, radiation, salt, and other stresses. 2nd edition. Academic Press, Inc. London.

Linsmaier, E.M., and Skoog, F. 1965. Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. 18:100-127.

Lloyd, G., and McCown, B. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb. Proc. Int. Plant Prop. Soc. 30:421-427.

Luckwill, L.C. 1970. The control of growth and fruitfulness in apple trees. In Physiology of Tree Crops. Edited by L. C. Luckwill and C. V. Cutting. Academic, New York. pp. 237-253.

Mahadeva, S. 1985. Mechanical harvesting of saskatoon fruit. WCSH. Reports of proceedings of the 41st annual meeting. pp.76-78.

Mazza, G. 1989. Saskatoons, new product opportunities. Saskatoon Production Manual. Prairie Fruit Growers Association.

McClelland, M.T., and Smith M.A.L. 1990. Vessel type, closure, and explant orientation influence of five woody species. HortScience 25:797-800.

McTavish, B. 1986. Seed propagation of some native plants is surprisingly successful. American Nurseryman 164:55-57.

Miller, W.S., and Stushnoff, C. 1970. A description of *Amelanchier* species in regard to cultivar development. Fruit Var. Hort. Digest 25:3-10.

Milliken, G.A., and Johnson, D.E. 1984. Analysis of messy data volume I : designed experiments. Van Nostrand Reinhold. New York, NY.

Murashige, T. 1977. Clonal crop through tissue culture. In Plant tissue culture and its biotechnological application, Proc. of the First Int. Congress on Medical Plant Research. Edited by W. Borz, E. Reinhard, and M.H. Zenk. U. of Munich, Springer-Verlag. Berlin. pp. 392-413.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nelson, S.H. 1987. Effects of stock plant etiolation on the rooting of saskatoon berry (*Amelanchier alnifolia* Nutt.) cuttings. Can. J. Plant Sci. 67: 299-303.

Nelson, S.H., and Bishop, B.H. 1980. Rootstock studies on saskatoons (*Amelanchier alnifolia* Nutt.) Can. J. Plant Sci. 60:879-882.

Nitsch, J.P., Nitsch, C., and Hamon, S. 1968. Realisation experimentale de l'androgenese chez divers Nicotiana. C.R. Seances Soc. Biol. Paris. 162:369-380.

Noiton, D., Vine, J.H., and Mullins, M.G. 1992. Effects of serial subculture *in vitro* on the endogenous levels of indole-3-acetic acid and abscisic acid and rootability in microcuttings of "Jonathan" apple. Plant Growth Regulation 11:377-383.

Norman, S.M., Poling, S.M., and Maier, V.P. 1988. An indirect enzyme-linked immunosorbent assay for (t)-abscisic acid in citrus, ricinus, and xanthium leaves. J. Agric. Food. Chem. 36:225-230.

Olson, R.A. 1984. Structural aspects of pollination in *Amelanchier alnifolia* (Maloideae). Can. J. Bot. 62:858-864.

Olson, A.R., and Steeves, T.A. 1982. Structural changes in the developing fruit wall of *Amelanchier alnifolia*. Can. J. Bot. 60:1880-1887.

Olson, A.R., and Steeves, T.A. 1983. Frost damage in flowers and immature fruits of *Amelanchier alnifolia* Nutt. (Maloideae). Can. J. Plant Sci. 63:461-466.

Parry, A. D., and Horgan, R. 1991. Physico-chemical methods in ABA research. In Abscisic Acid: Physiology and Biochemistry. Edited by W. J. Davies and H. G. Jones. Bio Scientific Publishers. Oxford. pp. 5-21.

Parry, A.D., and Horgan, R. 1992. Abscisic acid biosynthesis in higher plants. In Progress in Plant Growth Regulation. Edited by C.M. Karssen, L.C. Van Loon, and D. Vreugdenhil. Kleiwer Academic Publishers. Netherlands. pp. 160-172.

Phillips, I.D.J., and Wareing, P.F. 1958. Studies in dormancy of sycamore I. Seasonal changes in the growth-substance content of the shoot. J. Exp. Bot. 9:350-364.

Phillips, I.D.J., and Wareing, P.F. 1959. Studies in dormancy of sycamore. II. The effect of daylength on the natural growth-inhibitor content of the shoot. J. Exp. Bot. 10:504-514.

Powell, L.E. 1987. The hormonal control of bud and seed dormancy in woody plants. In Plant Hormones and Their Role in Plant Growth and Development. Edited by P.J. Davies. Martinus Nijhoff Publishers, Dordrecht. pp. 539-552

Pruski, K. 1987. Micropropagation of cultivars of the saskatoon *A. alnifolia* Nutt. M. Sc. thesis, Dept. of Plant Sc., U. of Alberta, Edmonton, Canada.

Pruski, K., Nowak, J., and Grainger, G. 1990. Micropropagation of four cultivars of saskatoon-berry (*Amelanchier alnifolia* Nutt.) Plant Cell, Tissue and Organ Culture 21:103-109.

Rodriguez, A., and Sanchez-Tames, R. 1986. Dormancy and seasonal changes of plant growth regulators in hazel buds. Physiol. Plant. 66:288-292.

Rodriguez, A., Canal, M.J., and Sanchez-Tames, R. 1991. Seasonal changes of plant growth regulators in *Corylus*. J. Plant Physiol. 138:29-32.

Ronald, W.G. 1989. What you should know about saskatoon berry production. Saskatoon Production Manual. Prairie Fruit Growers Association.

Ross, G., Elder, P.A., McWha, J.A., Pearce, D., and Pharis, R.P. 1987. The development of an indirect enzyme linked immunoassay for abscisic acid. Plant. Physiol. 85: 46-50.

Samish, R.M. 1954. Dormancy in woody plants. Ann. Rev. Plant Physiol. 5:183-204.

SAS Institute, Inc. 1985. SAS user's guide: Statistics. 5th ed. SAS Institute, Inc. Cary, N.C.

Seabrook, J.E.A. 1980. Laboratory culture. In Plant tissue culture as a source of biochemcials. Edited by V. Staba. CRC Press Inc. Boca Raton, Florida. pp. 1-18.

Seidlova, F., Kohli, R., Pavlova, L. 1981. Effects of abscisic acid on the growth pattern of the shoot apical meristem and on flowering in *Chenopodium rubrum* L. Ann. Bot. 48:777-785.

Seidlova, F. 1985. Growth regulators in changing apical growth at transition to flowering. Biol. Plant. 27:350-359.

Seeley, S. 1990. Hormonal transduction of environmental stresses. HortScience 25:1369-1376.

Seeley, S.D., and Powell, L.E., Jr. 1981. Seasonal changes of free and hydrolysable abscisic acid in vegetative apple buds. J. Amer. Soc. Hort. Sci. 106:405-409.

Smith, M.A.L. and McClelland, M.T. 1991. Gauging the influence of *in vitro* conditions on *in vivo* quality and performance of woody plants. *In Vitro* Cell Dev. Biol. 27:52-56.

St. Pierre, R.G. 1989. Magnitude, timing, and causes of immature fruit loss in *Amelanchier alnifolia* (Rosaceae). Can. J. Bot. 67:726-731.

St. Pierre, R.G. 1991a. Understanding flower and fruit production in the saskatoon. Horticultural Research and Extension Bulletin. Dept. of Horticulture Science, University of Saskatchewan.

St. Pierre, R.G. 1991b. Growing saskatoons - A manual for orchardists. Horticultural Research and Extension Bulletin. Dept. of Horticulture Science, University of Saskatchewan.

St. Pierre, R. 1992. The development of native fruit species as horticultural crops in Saskatchewan. HortScience 27:866 and 947.

St. Pierre, R.G., and Steeves, T.A. 1990. Observations on shoot morphology, anthesis, flower number, and seed production in the saskatoon, *Amelanchier alnifolia* (Rosaceae). Can. Field-Nat. 104:379-386.

Steeves, M.W., and Steeves, T.A. 1990. Inflorescence development in *Amelanchier alnifolia*. Can. J. Bot. 68:1680-1688.

Struve, D.K., and Lineberger, R.D. 1985. Field transplant survival of *Amelanchier* liners produced by tissue culture. Plant Propagator 31:11-13.

Torres, K.C. 1989. Tissue culture techniques for horticultural crops. Van Nostrand Reinhold. New York.

Turner, N.J., and Szczawinski, A.F. 1979. Edible wild plants of Canada. No. 3. Edible wild fruits and nuts of Canada. Canada National Museum. National Museum of Natural Sciences. pp.132-137.

Vegis, A. 1964. Dormancy in higher plants. Ann. Rev. Plant Physiol. 15:185-224.

Walker-Simmons, M. 1987. ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol. 84:61-66.

Walker-Simmons, M.K., and Abrams, S.R. 1991. Use of ABA immunoassays. In Abscisic Acid: Physiology and Biochemistry. Edited by W. J. Davies and H. G. Jones. Bios Scientific Publishers. Oxford. pp. 53-61.

Wallace, J.A., and Graham, T.O. 1976. Outlook in western Canada towards improvement of the genus *Amelanchier*. WCSH. Reports of the proceedings of the 32nd annual meeting. pp. 81-107.

Walton, D.C. 1987. Abscisic acid biosynthesis and metabolism. In Plant Hormones and Their Role in Plant Growth and Development. Edited by P.J. Davies. Martinus Nijhoff Publishers, Dordrecht. pp. 113-131.

Wareing, P.F. 1956. Photoperiodism in woody plants. Ann. Rev. Plant Physiol. 7:191-214.

Wareing, P.F., and Saunders, P.F. 1971. Hormones and dormancy. Ann. Rev. Plant Physiol. 22:261-288.

Webber, J.E., Laver, M.L., Zaerr, J.B., and Lavender, D.P. 1979. Seasonal variation of abscisic acid in the dormant shoots of Douglas-fir. Can. J. Bot. 57:534-538.

Weiler, E.W. 1984. Immunoassay of plant growth regulators. Ann. Rev. Plant. Physiol. 35:85-95.

Williams, S. 1994. Commercial saskatoon berry production on the prairies - A growers' guide. University Extension Press, Extension Division, University of Saskatchewan.

Wood, B.W. 1983. Changes in indoleacetic acid, abscisic acid, gibberellins, and cytokinins during budbreak in pecan. J. Amer. Soc. Hort. Sci. 108:333-338.

Wright, S.T.C. 1975. Seasonal changes in the levels of free and bound abscisic acid in Blackcurrent (*Ribes nigrum*) and Beech (*Fagus sylvatica*) buds. J. Expt. Bot. 26:161-174.

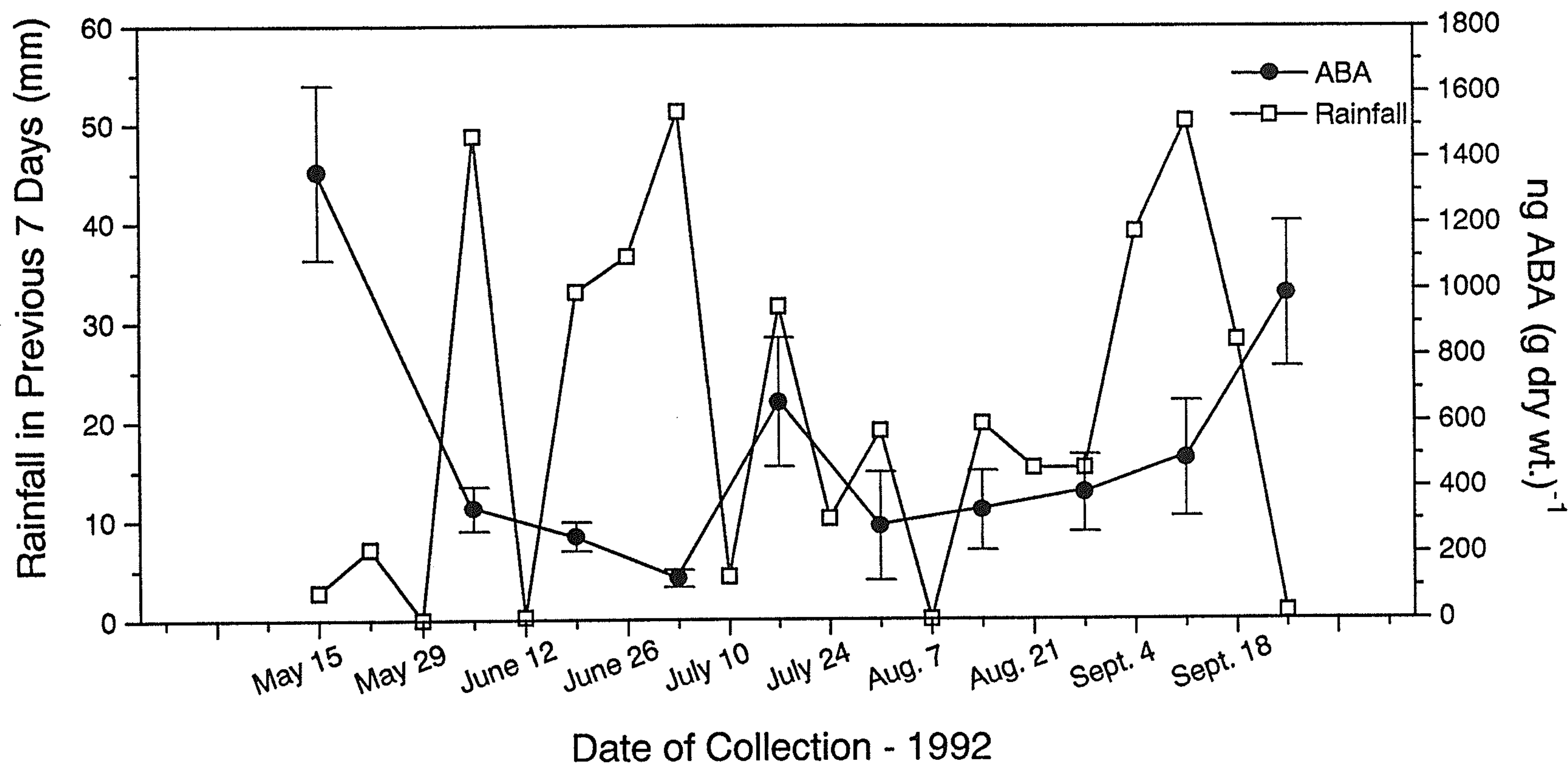
Zeevaart, J.A.D., and Creelman, R.A. 1988. Metabolism and physiology of abscisic acid. Ann. Rev. Plant Physiol. Plant. Mol. Biol. 39:439-473.

APPENDICES

Appendix A: Comparison of ABA concentrations between two methods of handling excised shoot tips.

Shoot tips were excised in the field and either frozen immediately in liquid nitrogen or transported back to the lab in a cooler to determine if ABA concentrations of the later would be elevated due to the stress of handling. A Student's t-test was used to compare the concentrations of ABA between the two methods of handling shoot tips and a p value of 0.5469 was obtained. Thus, there was no significant difference in ABA concentrations between shoot tips that were frozen immediately in liquid nitrogen and those that were transported back to the lab in a cooler. Increased ABA due to stress was not apparent in shoot tips transported in a cooler and, therefore, this method of handling shoot tips was considered to be acceptable.

Appendix B. The relationship of ng ABA (g dry weight)$^{-1}$ to rainfall in the 7 previous days (mm) during the 1992 study season.



Appendix C. The relationship of ng ABA (g dry weight)$^{-1}$ to mean weekly temperature (^{o}C) during the 1992 study season.