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THE UNIVERSITY OF ALBERTA

Metabolic studies of the mechanisms underlying age-reduced growth potential of potato (*Solanum tuberosum* L.) seed-tubers.

by

Loretta J. Mikitzel (C)

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF **Doctor of Philosophy**

IN

Horticulture

Department of Plant Science

EDMONTON, ALBERTA
Fall 1989

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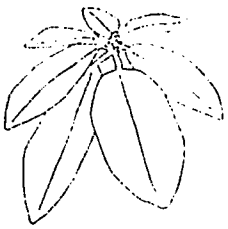

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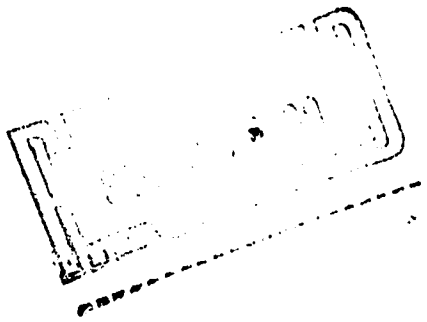

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.....

Supervisor

.....

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.....

Date: 12 Oct 1987

To my husband, Robert, for his gifts of love and laughter.

ABSTRACT

Metabolic processes associated with sprout growth were studied in potato seed-tuber tissue after long-term storage, to identify underlying mechanisms of the age-induced loss of sprout vigor. As seed-tuber age was increased from 5 to 19 months, apical dominance between and within eyes was lost. Shoot, root and leaf dry weights, and leaf number and area of individual plants from older tubers were reduced (up to 81%), compared with the single plant from younger tubers. Also, plants from older tubers partitioned more dry matter into stems relative to leaves and roots. These effects reflect age induced changes in plant morphology and vigor. Treating tissue from older tubers with auxin restored apical dominance. Auxin also altered morphology of the plants from older tubers to more closely resemble that from younger tubers, by increasing root and leaf weights and leaf area per leaf. Plant vigor however, was only partially restored, suggesting that an auxin imbalance in the tuber tissue is not solely responsible for the age-induced loss of growth potential.

Studies on tuber carbohydrate and nitrogen (N) reserves during sprouting revealed significant age related differences in the efficiency with which these reserves were mobilized. The rates of translocation apparently were not influenced by tuber age. During sprouting, reducing sugars accumulated twice as fast in older tubers than in younger tubers. Concomitant decreases in sucrose levels in older tubers implied less efficient conversion of reducing sugars to sucrose (the main translocatable carbohydrate) or higher rates of sucrose hydrolysis. In aged tubers, it appears that the amount of carbohydrate available in the translocatable form is limiting plant growth and not the absolute amount potentially available. Interconversions between the tuber N fractions during sprouting depended upon tuber age. The insoluble:soluble protein N ratio of older tuber tissue decreased 1.7 fold faster than that of younger tubers during sprout development. Increased loss of protein to the insoluble (and therefore immobile) N fraction in older tubers reflects altered N metabolism during sprouting, and significantly lower amounts of total N were found in the shoots. Reduced vigor of shoots from older tubers was also closely related to increased competition among the multiple shoots for available carbohydrate and N reserves and lower sink strength of the collective sprouts compared with that of the single sprout produced by younger tubers.

Advanced tuber age coincided with stronger expression of the alternative (alt) respiratory pathway, possibly due to age-induced changes of the tissue with increasing internal ethylene concentration. During sprouting, the rate of alt respiration of older tubers increased to levels 30-fold greater than that of younger tubers. Cytochrome (Cyt)-mediated respiration was not affected by tuber age or sprouting, averaging 18 nmol O_2/min/g frest weight. Sprouting older tubers, through the activity of the cyt-mediated and alt pathways, generated more energy than did younger tubers. The increased energy metabolizing ability of older tubers was, however, less efficient at meeting the demands of sprout growth, as evidenced by the lower dry weight of sprouts produced. Apparently, processes not directly involved in sprout growth (eg. altered carbohydrate metabolism) require additional energy in older tubers.

Polyamine (PA) metabolism was also influenced by tuber age. Putrescine (Put) increased and spermidine (Spd) and spermine (Spm) decreased with advancing tuber age to 31 months. During sprouting, the Put, Spd and Spm contents of older tuber tissue was low, a reflection of the 45 % decline in ornithine decarboxylase activity with time. Reduced growth potential of older tubers was correlated with lower PA content of the tubers.

Data presented here show that prior to and during plant establishment, tuber age has a major influence on auxin and polyamine metabolism, respiratory activity and reserve mobilization.

ACKNOWLEDGMENTS

I am grateful to my supervisor, N. Richard Knowles, for his friendship, patience, and encouragement during the course of the study.

Special thanks go to Dr. J. Hoddinott and Dr. A. Horak for their critical review of this work, and to Dr. R. Houtz who acted as external examiner.

I also wish to thank Mr. G. Botar for his help in preparing this manuscript.

Finally, I wish to acknowledge the financial support which made this research possible, NSERC grants to NRK and an NSERC scholarship to L.JM.

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LIST OF ABBREVIATIONS

ACC	1-aminocyclopropane-1-carboxylic acid
ADC	arginine decarboxylase
alt	alternative
cyt-mediated	cytochrome-mediated
DAP	days after planting
DTT	dithiothreitol
LAR	leaf area ratio
N	nitrogen
ODC	ornithine decarboxylase.
PA	polyamine
PCA	perchloric acid
PMSF	phenylmethylsulfonyl fluoride
Put	putrescine
PVP	polyvinylpyrrolidone
res	residual
RGR	relative growth rate
RH	relative humidity
SAM	S-adenosylmethionine
SAMDC	S-adenosylmethionine decarboxylase
SHAM	salicylhydroxamic acid
Spd	spermidine
Spm	spermine
ULR	unit leaf rate

CHAPTER I

BACKGROUND AND OBJECTIVES

Potatoes (*Solanum tuberosum* L.), unlike most other major food crops, are vegetatively propagated. Seed-tubers possess meristems which develop into daughter plants and yield the subsequent crop. Provided that good agronomic practices are followed and seed-tubers are disease free (certified), the physiological age of the seed-tuber at planting is one of the most determinant factors influencing crop growth and yield. The physiological age of a seed-tuber is dictated by chronological age and the previous conditions of its storage environment (Madec, 1978). In most cases, the environment in which seed-tubers were grown has little effect on subsequent performance (Jones et al., 1981). There are therefore two methods by which physiological age of potato tubers may be manipulated - by elevating storage temperature over a constant time period or by altering storage time at a constant temperature.

Since 1925, it has been recognized that advanced seed-tuber age, whether induced chronologically or by elevated storage temperature, modifies the growth habit of potatoes (Bushnell, 1925). The most obvious growth response to advancing age is a gradual loss of apical dominance. Figure 1 illustrates the difference in growth from seedpieces of potato seed-tubers stored continually at 4°C (95 % RH) for 7 and 19 months. Seedpieces from the 7-month-old tubers produced a few vigorous shoots, each with a strong root system. Seedpieces from tubers stored for 19 months are displaying reduced growth potential, and this is reflected in a loss in apical dominance between and within nodes, less vigorous roots and generally greatly reduced vigor of individual shoots compared with that from 7 month-old tubers.

From a production standpoint, plants from aged seed-tubers emerge more rapidly, develop a greater number of mainstems, initiate tubers sooner and at a lower leaf area index, and senesce earlier in the growing season than plants from younger seed-tubers (Wurr, 1978; Bodlaender and Marinus, 1987; Kawakami, 1980). This behaviour is ultimately connected to crop performance - higher yield from the few, vigorous mainstems per seedpiece from younger seed-tubers and lower yield and grade from the many, less vigorous mainstems per seedpiece from older seed-tubers (Iritani et al., 1983). It is thus apparent that age of a seed-

Figure 1. Sprouting characteristics of commercial-size seedpieces from potato seed-tubers stored at 4°C for 7 (a) and 19 (b) months. The seedpieces were planted into 18.5-cm diameter pots containing a soil/peat/sand mix (1/1/1; v/v/v) and maintained in a controlled environment for 10 days.


b
a

tuber influences all stages of daughter plant growth, from sprouting to final yield. Hence, understanding the processes of aging in potato seed-tubers is of considerable horticultural importance.

In reference to plants, Leopold (1975) defined aging as the passive (non-regulated) degeneration of physiological function with the passage of time, which is primarily driven by external forces; and senescence to encompass the endogenously controlled processes which lead to death. Similar to the deterioration of true seeds (Roberts, 1988), the changes that result in reduced growth potential and ultimately death of a seed-tuber are probably a good example of the stochastic aging process. It is difficult, however, to specifically associate particular physiological changes with either aging or senescence since the active process of senescence is the final stage of aging. Hence, the terms aging and senescence have been used throughout this thesis and no attempt is made to distinguish between the two processes.

Although little is known about the change(s) in metabolism of meristems and associated tuber tissue during potato aging, much can be gleaned from studies on aging in other plant systems. For example, in true seeds aging results in an increase in germination time, a loss of seedling vigor (reduced height or leaf area) and an increase in the number of malformed plants (Priestly, 1986; Villiers, 1980). Many of the morphological effects of aging in true seeds are similar to those in seed-tubers, indicating similar types of deterioration with time.

At the cellular level, senescence entails a sequence of highly ordered events consisting of many sequential and parallel steps. The underlying triggering mechanism however, has yet to be established. In true seeds, age-induced loss of germinating ability and seedling vigor have been correlated with significantly reduced protein and nucleic acid synthesis (Sen and Osborne, 1977), altered respiratory metabolism (Leopold and Musgrave, 1980), loss of phospholipid and increased gel-phase lipid in the membranes (Senaratna et al., 1988), and increased efflux of cytoplasmic solutes (Priestly, 1986). These observations strongly suggest that membrane integrity is gradually lost with advancing seed age, and impaired membrane function may be the root of reduced seedling vigor.

Even though the influence of seed-tuber age on plant growth and yielding ability has been investigated extensively, studies dealing with the physiological and biochemical changes that occur during aging of potato seed tubers are lacking. This is surprising in light of the dramatic effect that seed tuber age has

on growth and yield potential. Accordingly, the research herein was initiated to identify physiological processes that are affected during aging of tuber tissues, and to relate age-induced alterations in metabolism to reduced growth potential.

The Objectives were 4-fold:

1. Study the effect of auxin in restoring vigor and plant morphology, after characterizing the effect of seed-tuber age on plant growth and dry matter partitioning.
2. Characterize the efficiency of seed-tuber carbohydrate and nitrogen reserve mobilization during sprouting as affected by seed-tuber age.
3. Determine age-linked differences in the relative contribution of alternative and cytochrome-mediated respiration of seed-tubers during early sprout development.
4. Examine polyamine metabolism during long-term storage and the onset of sprout growth.

Tubers that have been subjected to long-term chronological aging are ideal for fundamental studies of the aging process because these tubers produce consistent effects on plant growth and development, with which physiological differences can be correlated. Hence, to maximize longevity, seed-tubers were aged chronologically at a constant temperature of 4°C and studies were conducted on tubers which differed in age by at least 12 months.

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CHAPTER II

THE EFFECT OF POTATO SEED-TUBER AGE ON PLANT ESTABLISHMENT AND AMELIORATION OF AGE-LINKED EFFECTS WITH AUXIN

Introduction

Prior to emergence, sprouts are solely dependent upon reserves stored within the potato (*Solanum tuberosum* L.) seed-tuber for growth. Moreover, early sprout vigor significantly influences yielding ability (Toosey, 1963). Seed-tuber physiological age, a somewhat subjective measure of the developmental status of a tuber, is a major factor in determining the number and vigor of shoots produced upon sprouting. The effect of age on plant growth from single-eye potato seedcores taken from seed-tubers which differed in age by 12 months is illustrated in Table 1. Seedcores from younger seed-tubers characteristically produced one vigorous, apically dominant sprout, while those from older seed-tubers, having lost apical dominance, developed multiple sprouts per eye. As sprout number increases with advancing tuber age, vigor (dry matter accumulation per unit time) of individual sprouts declines (Knowles, 1987; Mikitzel and Knowles, 1989ab). Though absolute values vary depending upon growth period and duration of storage (chronological age), the general trends in age-reduced apical dominance and vigor are consistent.

Storage time and temperature interact to dictate the physiological age which in turn influences the length of the incubation phase of potato seed-tubers. The incubation phase is the period between the onset of sprouting, and tuber initiation on the developing sprouts (Madec and Pérennec, 1955). Even at storage temperatures which are inhibitory to sprouting (below approximately 5° C), tubers which have entered the incubation phase continue to age and sprout growth occurs at a very slow rate (Krijthe, 1962; Madec, 1978). The length of the incubation phase is temperature dependent, with low temperatures prolonging the incubation phase and higher temperatures accelerating it (Hartmans and Van Loon, 1987). Hence, a given physiological age can be produced by manipulating storage temperature and/or length of the storage period.

Many of the age-induced alterations in potato plant growth appear to be auxin related. For example, treatment of 20-month-old potato seed-tubers with auxin decreased the number of stems produced per seedpiece and increased the

Table 1: Comparison of stem number, shoot dry weight and root dry weight of plants grown 24-30 days from single - eye seedcores from potato seed-tubers (cv. Russet Burbank) stored 5 to 19 months at 4°C (95 % RH).

Yield Component	Seed-tuber Age (months)					
	Expt 1		Expt 2		Expt 3	
	5	17	6	18	7	19
stem#/core	1	5**	1	6**	1	17**
shoot dwt						
mg/core	1014	1019	1407	1222**	122	125
mg/stem	1014	196**	1407	208**	122	7**
root dwt						
mg/core	159	129**	305	223**	82	44**
mg/stem	159	25**	305	38**	82	3**

** F-value for the difference between ages was significant at the 0.01 level.

average tuber size and total yield (Knowles, et al., 1985). With advancing physiological age, the loss in apical dominance is first evident between eyes on the tuber, and eventually multiple sprouts are produced within each eye. Auxin is thought to play a key role in the regulation of apical dominance in many plants (Wareing and Phillips, 1970). Thus, sprouts developing from aged seed-tubers may have a reduced ability to synthesize and/or translocate auxin, or possibly an increased rate of auxin catabolism, resulting in loss of apical dominance and the multiple shoot phenomenon. Reduced ability of plants growing from older seed-tubers to develop roots (Table 1) may also be an expression of auxin imbalance, since auxin is involved in root growth and development in many species (Wareing and Phillips, 1970).

Despite several reports on the effects of advanced seed-tuber age on overall growth and yield, an in-depth study characterizing the effect of age on the partitioning of dry matter between plant yield components during early establishment is lacking. Such a study is a prerequisite to defining the mode of action in relation to age-reduced vigor of potato seed-tubers. In this study, we have characterized the way in which advanced seed-tuber age influences growth potential and morphology at the whole-plant level. Furthermore, we have demonstrated that the alterations in plant morphology due to advanced tuber age can be overcome by auxin. Thus, dysfunctions in auxin metabolism are implicated in affecting age-reduced growth potential of potato seed-tubers.

Materials and Methods

Tuber sampling

Potato (*Solanum tuberosum* cv. Russet Burbank) seed-tubers (PVX tested, Elite III) were harvested and stored at 4°C (95% RH) for 5 to18 months. These conditions totally inhibited sprouting. In preparation for growth studies, tubers were acclimated to room temperature for 24 h in the dark and then blocked for size. Single-eye seedcores for the various studies were cut with a cork borer (1.8 cm diameter) from the middle portion of the tubers, perpendicular to the long (apical to basal) axis and trimmed to a length of 2 cm. Eyes on the apical and basal portions were avoided. The cores were rinsed in distilled water prior to planting.

Growth Characterization Study

Seedcores from 6 and 18-month-old seed-tubers were planted into 15-cm diameter pots (3 seedcores per pot) containing a mixture of soil, peat and sand (2:3:3; v/v/v). The pots were placed in a growth chamber with 25/18°C day/night temperatures in a randomized complete block design with 10 treatments (2 seed-tuber ages X 5 harvest dates) and 6 blocks. A combination of cool white fluorescent and incandescent bulbs provided 450 μmol m^{-2} s^{-1} light intensity for 16 h per day. Pots were watered as needed, fertilized at weekly intervals after emergence (250 ml per pot of 1 g/l 20:20:20 soluble fertilizer), and the number of days to emergence was recorded. Plants were harvested 10, 15, 20, 25 and 30 DAP. At each harvest, plants were divided into stems, leaves and roots. Stem number, leaf number and leaf area were recorded. Plant tissues were dried separately at 80°C for 72 h and dry weights recorded. RGR (1/W x dW/dt), ULR (1/LA x dW/dt) and LAR (LA/W) were also calculated (W = plant dry weight, dW/dt = the change in plant dry weight per unit time).

Auxin Study

Single-eye seedcores from 5 and 17-month-old seed-tubers were immersed in a solution of 0.1% (w/v) Tween 20 containing 0, 50, 100 or 150 mg/l NAA for 5 min. The treated cores were then air-dried for approximately 10 min and planted (3 per pot) into medium in 15-cm diameter pots as described above. The pots were arranged in a randomized complete block design with 8 treatments (2 seed-tuber ages X 4 NAA concentrations) and 6 blocks under the growth conditions already described. The plants received 250 ml of a 1 g/l solution of 20-20-20 soluble fertilizer weekly, starting 21 DAP. The plants were harvested 32 DAP, and the various yield components were handled as in the Growth Characterization Study.

Statistical Procedures

For each study, the plant growth data were subjected to analyses of variance and, where appropriate, sums of squares were partitioned into individual degrees of freedom components of both main effects and interactions. Regression analysis was used to fit polynomial models to the data.

Results

Growth Characterization Study

In this study, seed-tuber age apparently had no effect on time to emergence, and by 10 DAP 100 % emergence was recorded. A single shoot emerged from each 6-month-old seedcore. In contrast, seedcores from 18-month-old seed-tubers produced an average of 6 shoots, reflecting loss in apical dominance with advanced age. The data in Figure 1 illustrate, on an individual shoot basis, the influence of tuber age on various plant yield components over time. Shoot (Fig. 1a) and root (Fig. 1b) dry matter increased quadratically for both ages of seedcores; however, the rates of growth were significantly lower from the older seedcores. By the end of the study, each plant from the older cores had accumulated 81% and 79% less shoot and root dry matter respectively, compared with that from younger cores.

Leaf number (Fig. 1c) and area per shoot (Fig. 1d) were also influenced by tuber age. Leaf number increased linearly with time and the rate of leaf production on each shoot from younger seedcores ($Y_{\square} = -1.3+0.6X$) was 2-fold greater than that from older seedcores ($Y_{\blacklozenge} = -1.2+0.3X$). Hence, at the end of the 30 d growth interval, individual shoots from 18-month-old seedcores yielded an average of 8 leaves compared with 16 on each plant from 6-month-old cores. Leaf area per shoot (Fig. 1d) was also significantly lower for plants from older compared with plants from younger seedcores throughout the study. Although leaf area per shoot increased quadratically for plants from both ages of seedcores, after 30 d of growth, leaf area of each shoot from 6-month-old cores was 4.2-fold higher than that of each shoot from 18-month-old seedcores, reflecting a highly significant age x time interaction. The maximum leaf area per leaf (Fig. 1d, inset), was reached at 25 DAP and, at that time, area of individual leaves on plants from 6-month-old cores was 2.9-fold greater than that from 18-month-old cores.

Leaf/stem dry weight ratio was calculated to assess the effect of tuber age on dry matter partitioning within the shoots. A significant ($P< 0.01$) age x time (quadratic) interaction in the leaf/stem dry weight ratio was characterized (Fig. 2). The maximum was reached 20 to 23 DAP where a 1.5-fold higher ratio was evident in shoots developing from younger cores compared with those from older seedcores. At 10 DAP, the leaf/stem dry weight ratio was 0 for plants from 18-month-old seedcores, due primarily to emergence and growth of stems in the absence of leaf expansion.

Figure 1. Shoot and root growth, and leaf development of individual plants from single-eye seedcores from 6 (□) and 18-month-old (◆) potato seed-tubers. F-values for the age x time interactions were significant at the 0.05 level (b) and (d, inset) and at the 0.01 level for (a), (c) and (d).

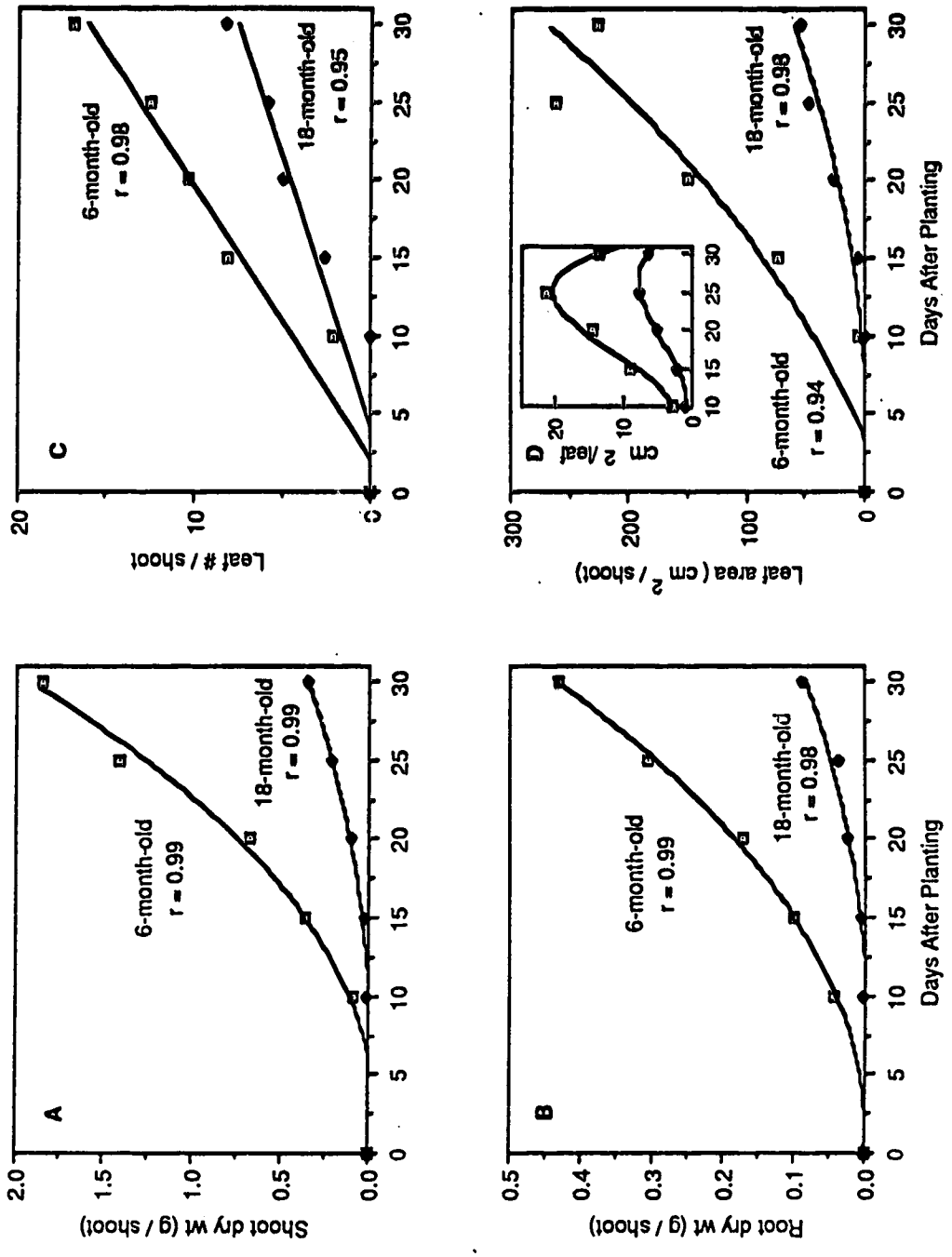
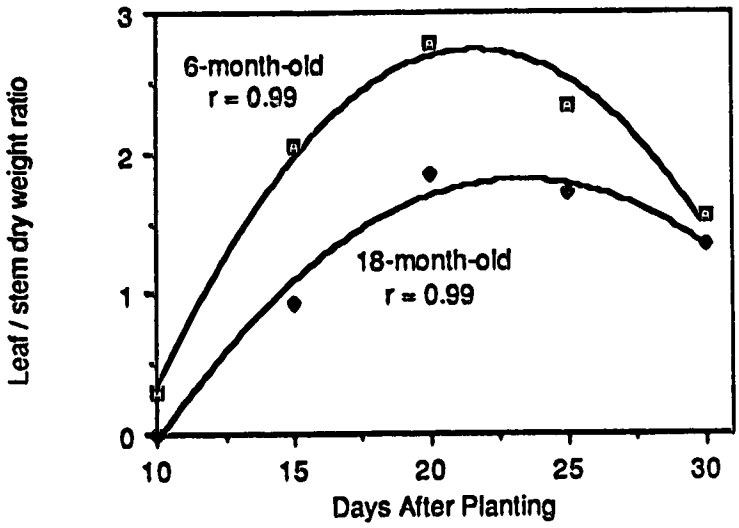

A
Shoot dry wt (g / shoot)
6-month-old
r = 0.99
18-month-old
r = 0.99
B
Root dry wt (g / shoot)
6-month-old
r = 0.99
18-month-old
r = 0.98
C
Leaf # / shoot
6-month-old
r = 0.98
18-month-old
r = 0.95
D
Leaf area (cm 2 / shoot)
cm 2 /leaf
6-month-old
r = 0.94
18-month-old
r = 0.98
Days After Planting
Days After Planting

Figure 2. Time course of leaf to stem dry weight ratio for plants from single-eye seedcores from 6 ( ) and 18-month-old ( ) potato seed-tubers. F-value for the interaction seedcore age x time (quadratic) was significant at the 0.01 level.


Leaf / stem dry weight ratio
3
2
1
0
6-month-old
r = 0.99
18-month-old
r = 0.99
10
15
20
25
30
Days After Planting

On a total seedcore basis, the RGR of plants from older cores was significantly greater than for plants from younger cores, even though ULR, a measure of the efficiency of the plant as a producer of dry matter, was equal for plants from both ages (Table 2). Plants from younger cores were initally more efficient at producing leaf area, as measured by LAR, than plants from older cores. By 15 DAP, LAR was equal for plants from both ages of seedcores and, with further growth, the LAR of plants from older cores surpassed that of plants from younger cores and remained 10-26% higher for the duration of the study.

The polygonal diagrams of Figure 3 represent, on a total seedcore basis, the influence of seed-tuber age on the development of yield components during early plant establishment. The influence of seed-tuber age on growth and whole plant morphology is thus illustrated. The results of the analysis of variance for each of the yield components displayed in Figure 3 is summarized in Table 3. Seed-tuber age significantly affected all parameters measured. The effect of age on apical dominance was evident as early as 10 DAP. Stem dry matter increased linearly for plants from both ages of seedcores, and collectively, the stems from older cores grew 12 % faster ($P< 0.01$) than those from younger cores. Compared with the single stem from 6-month-old seedcores, the multiple stems from 18-month-old cores accumulated 80 mg more dry matter by 30 DAP; a small but significant difference (Table 3).

The increase in leaf number per core was the second age-linked growth effect manifested early in the study. By 30 DAP, plants from older seedcores averaged a total of 46 leaves compared with 18 leaves produced by plants from younger cores, resulting in a significant age x time interaction. Similarly, the accumulation of leaf dry matter with time resulted in a significant age x time interaction. At all harvest dates, leaf dry weight for plants from older cores was significantly less than that for plants from younger cores, even though the rate of leaf dry matter accumulation for plants from older cores was 1.8 fold greater ($P<0.01$) than that of plants from younger cores over the study interval.

Leaf area per core also increased quadratically with time and significant differences existed between the plants from the two ages of seedcores. Initially (up to 15 DAP), leaf area of plants from younger cores expanded more rapidly, reaching a level 40% greater than that from older cores. By 20 to 25 DAP, leaf area was similar for plants from both ages of seedcores; however, by 30 DAP leaf area of plants from older cores exceeded that from younger cores by 26 %.

Table 2: Growth analysis of plants originating from single-eye seed-cores from 6 and 18-month-old potato seed-tubers. Growth indices were calculated based upon the total plant dry weight per core.

	ULR[1] mg/cm^2.d		LAR[2] cm^2/mg		RGR[3] g/g.d	
DAP[4]	Tuber age (months)					
	6	18	6	18	6	18
10	-	-	0.06	0.01	-	-
15	0.88	0.95	0.14	0.14	0.13	0.15
20	0.64	0.64	0.18	0.20	0.10	0.13
25	0.52	0.55	0.16	0.19	0.07	0.11
30	0.52	0.67	0.09	0.12	0.04	0.09
Age (A)[a]	ns[b]		ns		0.01	
DAP_{LT}	0.01		0.01		0.01	
DAP_{QT}	0.01		0.01		ns	
A X DAP_{LT}	ns		0.01		ns	
A X DAP_{QT}	ns		0.01		ns	

1, Unit leaf rate; 2, leaf area ratio; 3, relative growth rate; 4, days after planting. [a] Sources of variation (LT and QT subscripts indicate linear and quadratic trends, respectively). [b] Significance levels for indicated sources of variation.

Figure 3. Polygonal diagrams depicting growth and partitioning of assimilates for plants from single-eye seedcores from 6 and 18-month-old potato seed-tubers. DAP, days after planting. Key to axes appears on the 30 DAP polygonal diagram for each age of seed-tuber from which seedcores were taken. Summary of statistical analyses is presented in Table 3.

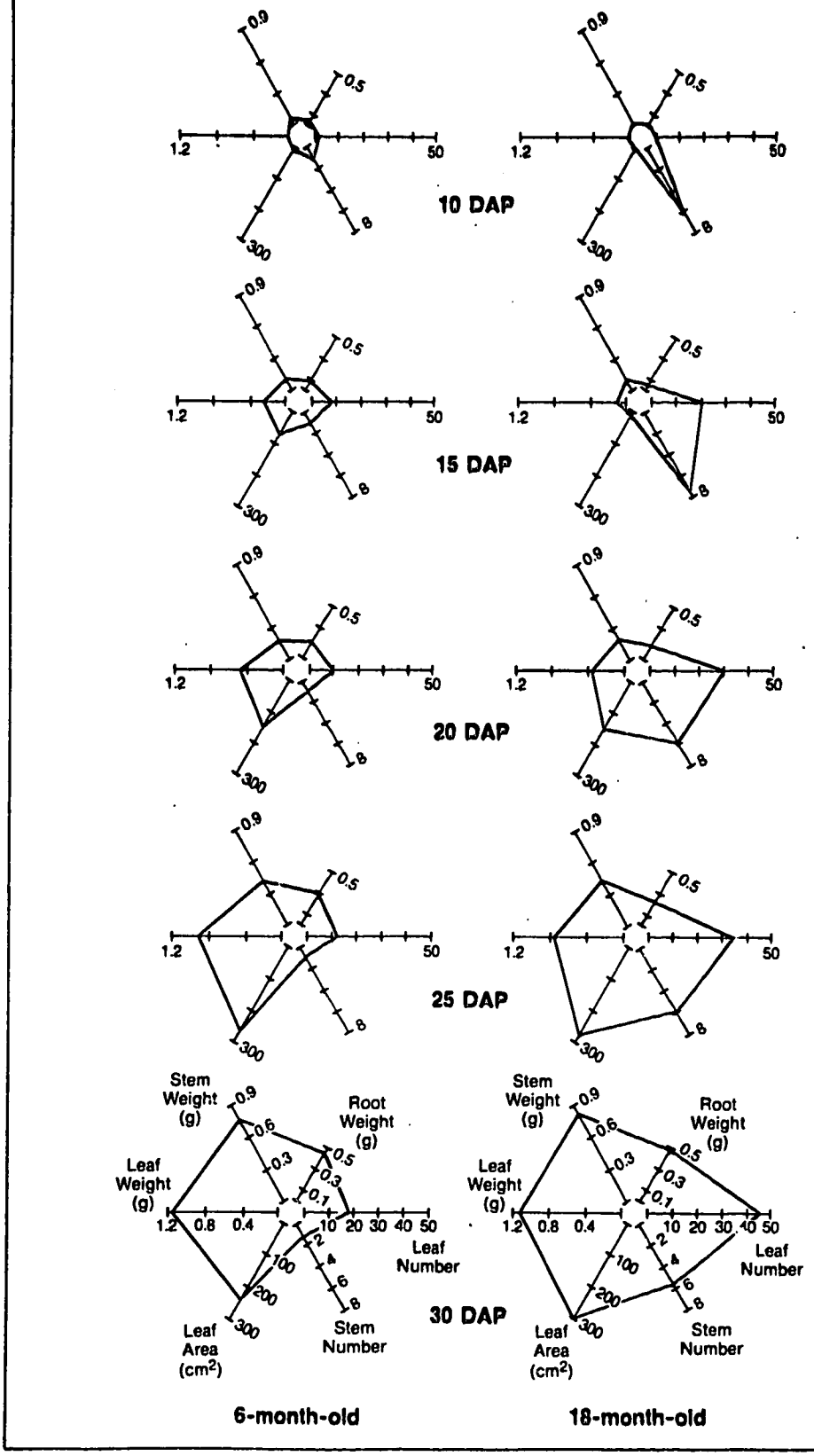

10 DAP
15 DAP
20 DAP
25 DAP
30 DAP
Stem Weight (g)
Root Weight (g)
Leaf Weight (g)
Leaf Number
Leaf Area (cm²)
Stem Number
6-month-old
18-month-old

Table 3: Summary of analyses of variance for growth and dry matter partitioning of plants from single-eye seedcores from 6 and 18-month-old potato seed-tubers. Plants were harvested 10, 15, 20, 25 and 30 days after planting. Values represent level of significance of F-values for indicated sources of variation. The final row reports the least significant difference of the mean (LSD) at the 0.05 level. Data are presented in Figure 3.

Source of Variation	stem number	leaf number	Dry Weight			leaf area
			stem	leaf	root	
Age (A)	0.01	0.01	0.05.	0.01	0.01	0.05
$Time_{LT}$	ns	0.01	0.01	0.01	0.01	0.01
$Time_{QT}$	ns	0.01	0.01	0.01	0.01	0.01
A X $Time_{LT}$	ns	0.01	0.01	ns	ns	0.01
A X $Time_{QT}$	ns	0.01	ns	0.01	0.05	0.01
$LSD_{0.05}$	0.97	4.06	0.05 g	0.07 g	0.05 g	16.5 cm^2

The root growth rate of plants from older cores was 41 % greater ($P < 0.01$) than that from younger cores yet, by 30 DAP no significant effect of seedcore age on root dry weight was evident.

Auxin Study

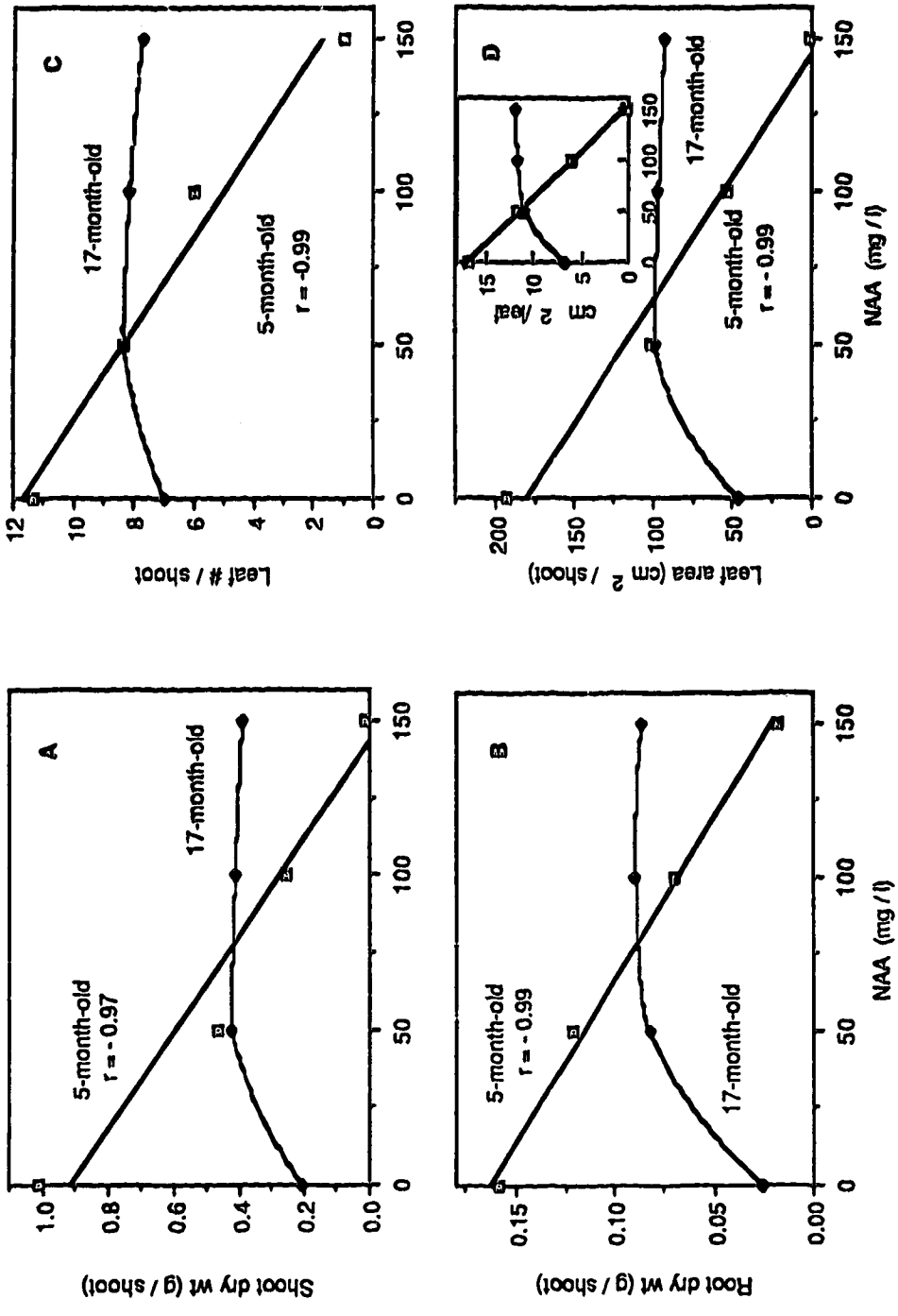
The potential of auxin to restore apical dominance and plant vigor was studied by pre-treating seedcores with NAA. In this study, the rate of plant emergence depended on both seed-tuber age and NAA concentration (results not shown). Although increasing the concentration of NAA delayed emergence of plants from both ages of seedcores, the effect on emergence from younger cores was much greater compared with that from older cores. Plants from untreated 5-month-old seedcores achieved 100 % emergence by about 10 DAP, 2.5 d later than those from 17-month-old controls. Treating 17-month-old seedcores with 50 and 100 mg/l NAA resulted in 100 % emergence by 10 DAP; however, 150 mg/l NAA delayed emergence by an additional 4 d. Treating 5-month-old seedcores with 50 and 100 mg/l NAA increased the time to 100 % emergence by 1.9 and 2.4-fold respectively, compared with controls. By 32 DAP, many of the 5-month-old seedcores treated with 150 mg/l NAA had failed to sprout.

Similar to the findings of Hartmans and Van Es (1979), shoot and root growth were either stimulated or inhibited by NAA treatment depending upon age of the tuber from which the seedcores were taken. In general, as NAA concentration increased, plant growth from 5-month-old cores was inhibited, and that from 17-month-old cores was stimulated.

Shoot (Fig. 4a) and root dry matter (Fig. 4b) accumulation of plants from younger cores were inversely related to NAA concentration . Decreases of 99% and 89 % in shoot and root dry matter, respectively, were obtained by treating 5-month-old cores with 150 mg/l NAA. Conversely, dry matter accumulation per shoot from 17-month-old seedcores treated with 50 mg/l NAA was increased by 2.1-fold over the controls and no additional increase in shoot dry weight occurred with greater NAA concentrations (Fig. 4a). Root dry matter of plants from 17-month-old cores was also increased maximally (3.2-fold over controls) by treatment with 50 mg/l NAA (Fig. 4b).

Leaf number per shoot of plants from 5-month-old cores decreased from 11.7 to 1.7 as the concentration of NAA increased from 0 to 150 mg/l (Fig. 4c). In contrast, plants from17-month-old seedcores averaged 7.8 leaves per shoot and

Figure 4. Shoot and root growth, and leaf development of individual plants from NAA-treated single-eye seedcores from 5 (□) and 17-month-old (◆) potato seed-tubers after 32 days of growth. F-values for the age x [NAA] interaction were significant at the 0.05 level for (a) and at the 0.01 level for (b), (c), (d) and (d, inset).


A
5-month-old
r = - 0.97
17-month-old
Shoot dry wt (g / shoot)
B
5-month-old
r = - 0.99
17-month-old
Root dry wt (g / shoot)
C
17-month-old
5-month-old
r = -0.99
Leaf # / shoot
D
17-month-old
5-month-old
r = - 0.99
Leaf area (cm 2 / shoot)
cm 2 /leaf
NAA (mg / l)
NAA (mg / l)

leaf number was unaffected by NAA. While 50 mg/l NAA decreased total leaf area per shoot by 34 % in plants from younger cores, it more than doubled leaf area per shoot in plants from older cores (Fig. 4d). Increasing the NAA concentration further reduced leaf area per shoot in plants from younger cores to nearly 0, but had no additional effect on leaf area per shoot of plants from older cores. Similar trends were observed in leaf area per leaf (Fig. 4d, inset). NAA affected a linear decrease in the area of individual leaves of plants from younger cores and at 50 mg/l, increased leaf area per leaf of plants from older cores by 62%.

Leaf/stem dry weight ratio (Fig. 5) for plants from untreated, 17-month-old seedcores was lower than that from 5-month-old control seedcores. This trend was also noted at 30 DAP in the Growth Characterization Study (Fig. 3). A significant linear decrease in leaf/stem dry weight ratio for plants from younger cores with increasing NAA concentration resulted in a ratio of only 0.2 at 150 mg/l NAA. In contrast, the leaf/stem dry weight ratio of plants from older cores reached a maximum at 50 mg/l NAA and the 17 % increase resulted in a ratio that was approximately equal to that for plants from the untreated, 5-month-old seedcores.

The polygonal diagrams of Figure 6 illustrate, on a total seedcore basis, the overall effects of tuber age and NAA concentration on plant growth and morphology. A summary of the analysis of variance for each yield component displayed in Figure 6 is presented in Table 4. Similarities between the shapes of the diagrams for the control plants in Figure 6 with those displayed at 30 DAP in Figure 3, demonstrate the consistency in the effect of advanced tuber age on plant morphology. Small changes in seed-tuber age (eg. from 5 to 6 months or 17 to 18 months) have little effect on plant form and, upon sprouting of seedcores, stem and leaf number were the yield components most noticeably altered by seed-tuber age.

With the highest concentration of NAA, the number of stems produced per 5-month-old core was reduced to 0.2, reflecting severe inhibition of sprouting. In the absence of NAA, 17-month-old cores produced an average of 5 stems and as NAA concentration increased, average stem number decreased to 1.8. Treating 5-month-old seedcores with increasing concentrations of NAA resulted in linear decreases in stem weight, leaf number, leaf dry weight, leaf area and root dry weight. Depending upon the yield component, the maximum inhibition ranged

Figure 5. Leaf to stem dry weight ratio after 32 days of growth of plants from NAA-treated single-eye seedcores from 5 (□) and 17-month-old (◆) potato seed-tubers. F-value for the interaction seedcore age x [NAA] was significant at the 0.01 level.

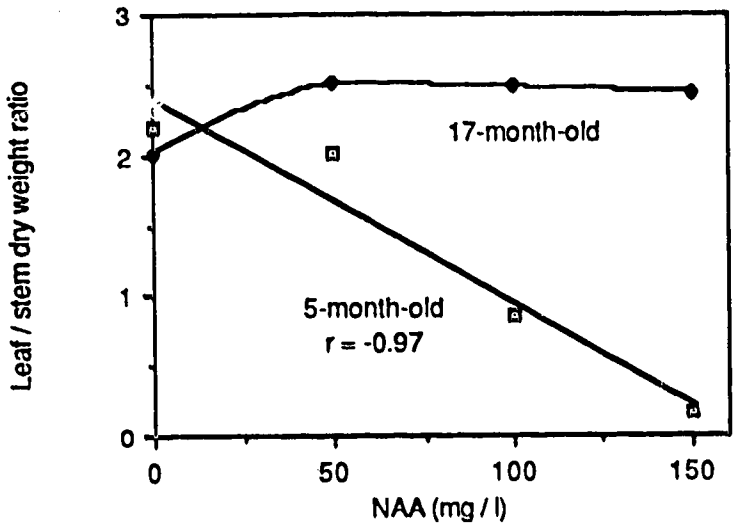

Leaf / stem dry weight ratio
3
2
1
0
17-month-old
5-month-old
r = -0.97
0
50
100
150
NAA (mg / l)

Figure 6. Polygonal diagrams depicting growth and partitioning of assimilates for plants grown 32 days from NAA-treated single-eye seedcores from 5 and 17-month-old potato seed-tubers. Prior to planting, seedcores were treated 5 minutes with 0, 50, 100 or 150 mg/l NAA. Key to axes appears on the polygonal diagram for 150 ppm NAA for both ages of seed-tubers from which seedcores were taken. Summary of statistical analyses is presented in Table 4.

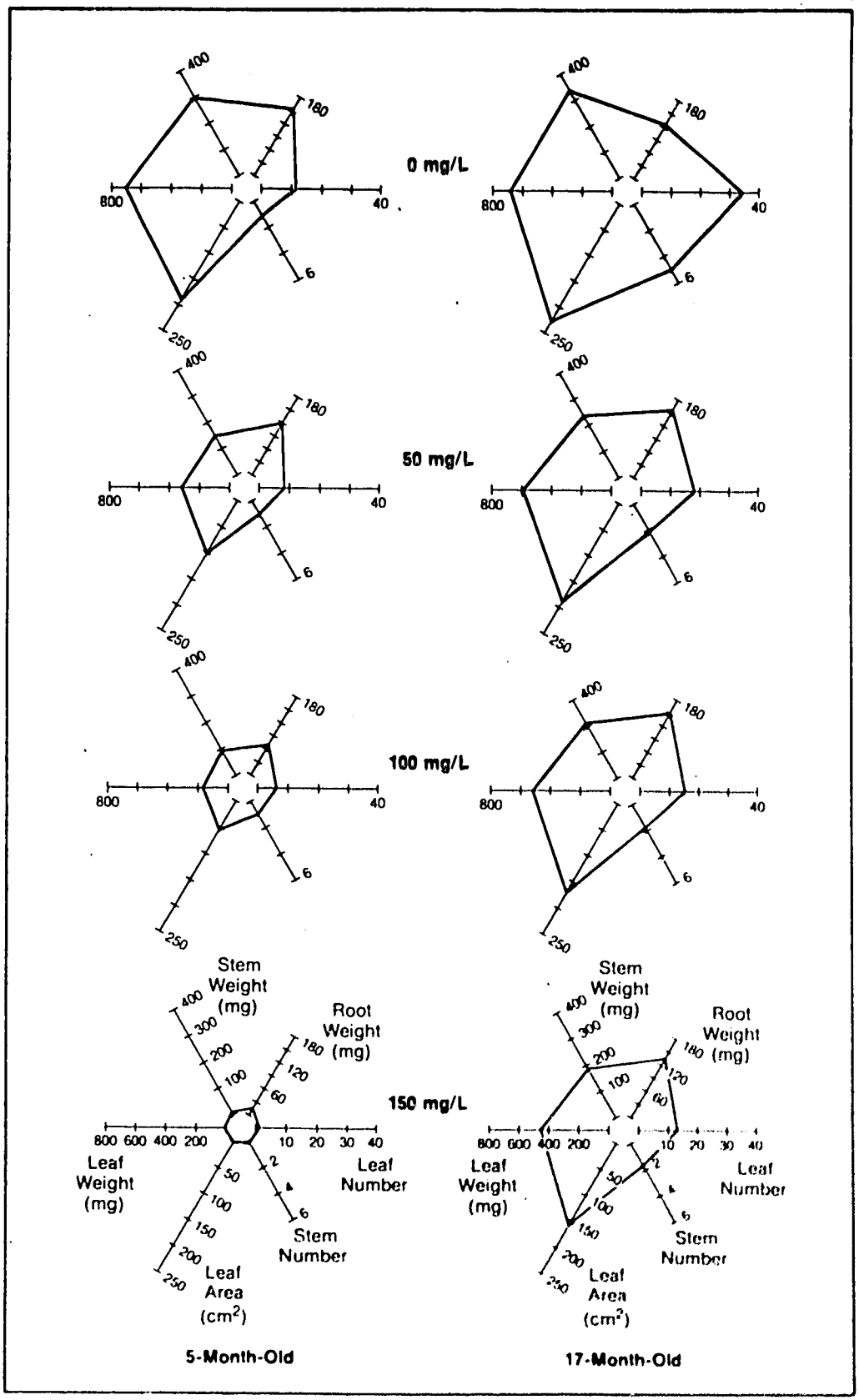

0 mg/L
50 mg/L
100 mg/L
150 mg/L
Stem Weight (mg)
Root Weight (mg)
Leaf Weight (mg)
Leaf Number
Stem Number
Leaf Area (cm²)
5-Month-Old
17-Month-Old

Table 4: Summary of analyses of variance for the effect of seed-tuber age and NAA on plant growth for plants grown (32 days) from single-eye seedcores from 5 and 17-month-old potato seed-tubers. Prior to planting, seedcores were treated 5 minutes with 0, 50, 100 or 150 mg/l NAA. Values represent level of significance of F-values for indicated sources of variation. Data are presented in Figure 6.

Source of Variation	stem number	leaf number	Dry Weight			leaf area
			stem	leaf	root	
Age (A)	0.01	0.01	0.01	0.01	0.01	0.01
$[NAA]_{LT}$	0.01	0.01	0.01	0.01	0.01	0.01
$[NAA]_{QT}$	0.05	0.05	ns	ns	ns	ns
A X $[NAA]_{LT}$	0.01	0.01	0.01	0.01	0.01	0.01
A X $[NAA]_{QT}$	0.01	0.01	ns	ns	ns	ns
$LSD_{0.05}$	0.76	4.50	0.08 g	0.16 g	0.05 g	50.2 cm^2

from 89 to 99 %. Clearly, exogenous NAA greatly suppressed growth from younger seedcores. With the exception of root dry weight, treatment of 17-month-old cores with NAA also resulted in linear reductions of the same yield components; however, the maximum inhibition was only 40 % for stem dry weight, 61 % for leaf number, 34 % for leaf dry weight and 32 % for leaf area. Root dry matter of plants from 17-month-old seedcores increased by 23 % in response to NAA treatment. Finally, treatment of seedcores from older seed-tubers with NAA altered the morphology of plants to more closely resemble that from untreated 5-month-old seedcores (compare the overall shape of the 17-month-old, 150 mg/l NAA polygonal diagram to that for the 5-month-old, 0 mg/l NAA diagram).

Discussion

In general, as seed-tuber age advances, time to emergence decreases. This has been attributed to growth in storage and thus the presence of sprouts at planting (Wurr, 1978; Madec, 1978). Younger seed-tubers, with very little or no sprout growth at planting, normally emerge later than older seed-tubers having extensive sprout growth. However, differences in plant emergence rates are not always found between seed-tubers of different ages. In the Growth Characterization Study, plants from seed-tubers that were stored for 6 and 18 months emerged simultaneously. If seed-tubers of different ages are desprouted immediately prior to planting, the age-related differences in emergence will be lost (Madec and Pérennec,1962). Similarly, if different ages of seed-tubers are desprouted and planted only after regrowth has started, age-related differences in emergence will not be evident (Bus and Schepes, 1978). Since there were no major differences in the degree of sprout development between the 6 and 18 month-old tubers used in the Growth Characterization Study, similar emergence rates were expected.

After 30 d of growth, total dry matter (stem plus leaf plus root dry weight) of plants from both 6 and 18-month old seedcores was equal (Fig. 3). Plants from older cores averaged 6-fold more stems and 3 fold more leaves than those from younger cores; however, proportional increases in the total stem and leaf dry weights did not occur. The total plant dry weight from older cores was thus divided among the multiple shoots and, as a result, each plant from older seedcores was only one-sixth as vigorous as the single plant from the younger seedcores (see Fig. 1). Furthermore, individual plants from the 18 month old

cores were not simply "miniatures" of those from the 6-month-old cores as evidenced by dissimilar dry matter partitioning. A larger proportion of dry matter was partitioned into stems of plants from older seedcores compared with those from younger seedcores (Fig. 3). Also, the increased production of leaves on plants developing from older seedcores occurred at the expense of total dry matter accumulation. Altered dry matter partitioning related to tuber age was also evidenced by greatly reduced leaf area per stem and per leaf of each shoot from older seedcores (Fig. 1d).

The calculated growth analysis parameters reflect the age-induced changes in plant form and vigor. The RGR of plants from both ages of seedcores decreased with time (Table 2). As the plants developed, RGR declined due to the increased amount of non-photosynthetic plant material (vascular, mechanical and other tissues) which was not directly contributing to further synthesis of new material. Differences between the RGR of plants from the 6 and 18-month-old seedcores reflect age-related differences in plant development and dry matter accumulation. Individual plants from 6-month-old seedcores accumulated significantly more dry weight than those from 18-month-old seedcores at each harvest date (Fig. 1a), and were therefore more advanced developmentally. The presence of larger quantities of vascular and structural tissues in the larger plants from the younger seedcores no doubt contributed to their lower RGR.

LAR, the ratio of assimilatory material per unit plant material present, was also dependent upon seedcore age (Table 2). Initially, plants from younger cores were more efficient at producing leaf area than plants from older cores. This is also shown by the leaf/stem dry weight ratio which was significantly lower for plants from the 18-month-old seedcores (Fig. 2). Over the latter part of the study, plants from older cores produced leaf area at a greater rate (higher LAR); however, leaf dry weight remained significantly lower compared with that from younger cores over the entire growth period (Fig. 3). Interestingly, ULR was similar throughout the study for plants from both seed-tuber ages (Table 2). Plants from both ages of seedcores were therefore equally efficient at producing dry matter, and the apparent increased efficiency with which plants from older seedcores produced leaf area (measured by LAR) did not translate into increased assimilatory efficiency. In other words, the greater number of leaves on plants from older cores are no more efficient at producing dry matter than the fewer leaves on plants from younger cores. Furthermore, each 18-month-old seedcore produced 6-fold more stems than each 6-month-old core. ULR for each stem

(which represents an individual plant) from the older seedcores was therefore reduced by a proportional amount.

The application of NAA to seedcores prior to planting somewhat ameliorated the age-induced changes in plant morphology and vigor. In the absence of NAA, plants from 17-month-old seedcores emerged earlier than those from 5-month-old seedcores. Russet Burbank seed-tubers stored at 4°C remain dormant for approximately 5 months. Hence, at the time of the experiment, the 5-month-old tubers were just emerging from dormancy and this resulted in delayed emergence compared with that from the 17-month-old tubers. The application of NAA apparently altered the natural hormonal balance within the meristems and seedcore tissue from both ages of tubers, and effectively delayed emergence compared with controls. Days to emergence of plants from older cores treated with intermediate auxin levels (50 and 100 mg/l) was equal to that of untreated younger cores. Hence, exogenous auxin may have been compensating for reduced levels of endogenous auxin in the older cores. Younger cores treated with 150 mg/l NAA appeared to be in a state closely resembling dormancy, as many had not emerged by 32 DAP. Cores from 5-month-old tubers treated with auxin displayed reduced sprouting ability and growth was greatly suppressed. This suggests that the exogenous NAA, in combination with endogenous auxin, resulted in supra-optimal levels of auxin within the younger seedcores and meristems, and the effect was either to prolong dormancy (Rapaport and Wolf, 1969) or to directly inhibit sprout growth (Wareing and Philip, 1970) to the extent where the seedcores appeared dormant. Roots arising directly from the seedcore tissue were observed only in younger cores treated with 150 mg/l NAA. Clearly, a hormonal imbalance existed within the treated younger cores which favored root growth over shoot growth.

Plants from 5-month-old seedcores were detrimentally affected by NAA treatment, in that all growth parameters were greatly reduced. On the other hand, plants from 17-month-old seedcores benefited from seedcore NAA treatment. Apical dominance of older cores was restored with NAA treatment and the vigor of individual plants was increased. For example, untreated seedcores from 17-month-old tubers produced an average of 5 stems with a total dry weight of 342 mg, or 66 mg per stem. With 150 mg/l NAA, the 1.8 stems produced per 17-month-old seedcore collectively weighed 185 mg, or 103 mg per stem. Although the total dry weight of stems produced from the older seedcores decreased after treatment with NAA, a 56 % increase in dry weight of individual stems was

realized. This relationship also holds true for leaf dry weight (77 % increase per leaf) and leaf area (75 % increase per leaf).

The number of stems produced by older cores treated with NAA approached that characteristic of untreated younger seedcores, indicating restoration of apical dominance. However, vigor of plants from older cores was not fully restored by treatment with NAA. Shoot and root dry matter, as well as leaf area per plant and per leaf, from older cores increased significantly in response to NAA, yet remained substantially lower than that from untreated younger seedcores (Fig. 4). Increased competition between multiple sprouts, together with decreased sink strength of individual sprouts for tuber reserves, has been implicated in contributing to the reduced vigor of older seed-tubers (Knowles, 1987). Auxin, by affecting apical dominance, decreased competition between sprouts, yet the full potential increase in sprout vigor was not realized. Even after auxin treatment, shoots from older seedcores were apparently less effective sinks for tuber reserves than the single shoot from younger seedcores. Also, increasing NAA concentration above 50 mg/l did not stimulate any additional growth response (positive or negative) from the older seedcores. Hence, an auxin imbalance is probably not solely responsible for the reduced growth potential of plants from aged seedcores. Age-reduced auxin levels, tissue sensitivity to auxin, and/or age-related metabolic dysfunctions in systems regulated by auxin may limit the response of older cores to auxin treatment.

Partial restoration of vigor to older tubers with exogenous auxin may be a reflection of age-induced limitations of the various biochemical processes which interact with auxin during sprouting of the tuber. For example, auxin stimulates both PA and macromolecular biosynthesis, two processes which are essential for, and correlated with, active plant growth (Galston and Kaur-Sawhney, 1987). It has been proposed that auxin acts through PAs in promoting growth, since hormone-mediated growth in many plant tissues requires PA synthesis prior to response. In dormant *Helianthus tuberosus* tubers (Bagni, et al., 1980) and parthenocarpic tomato ovaries (Mizrahi and Heimer, 1982), auxin-induced growth is preceeded by PA biosynthesis and is greatly reduced by inhibitors of the PA biosynthetic enzymes. Recently, reduced vigor of plants from aged potato seed-tubers has been correlated with significant reductions in ornithine decarboxylase (the enzyme involved in the first step of PA synthesis) activity and increases in Put titer of the tuber tissue during sprouting (Mikitzel and Knowles, 1989). During sprouting of 19-month-old seedcores, the increase in Put

over the initial titer was only 33% of that in seedcores from 7-month-old tubers. With increased tuber age PA metabolism was thus suppressed during sprouting, but not totally dysfunctional. Exogenous auxin application to older seedcores only partially restored vigor and the less than maximal response may be due to age-related alterations in the PA metabolic pathway. The possible relationships between PAs, auxin, potato tuber age and sprout vigor warrant further investigation.

From the data, it is apparent that advanced seed-tuber age results in loss of apical dominance and plant growth potential. Loss of growth potential with advanced age was characterized by reduced shoot, root and leaf dry weight, leaf number and area of individual plants from aged seedcores. Dry matter partitioning and thus plant morphology were also affected by tuber age, with a greater proportion of dry weight apportioned to stems at the expense of leaves and roots in plants from older seedcores. Auxin treatment of older seedcores restored apical dominance, and increased root growth, leaf dry weight and leaf area per plant. Auxin also altered the morphology of plants from older seedcores to more closely resemble that of younger cores.

Root growth of plants from older seedcores was stimulated by auxin, possibly contributing to enhanced vigor through increased mineral uptake. Although auxin did not fully restore growth potential to plants from aged seed-tubers, it is apparent that an age-induced auxin imbalance (or dysfunction in auxin metabolism) is a major contributor to the loss of sprout vigor that occurs and auxin is very important in governing plant morphology of potato.

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CHAPTER III

POTATO SEED-TUBER AGE AFFECTS MOBILIZATION OF CARBOHYDRATE AND NITROGEN RESERVES DURING PLANT ESTABLISHMENT[1]

Introduction

Vigor of a seed potato has been defined as the potential of the tuber to produce sprouts and plants under conditions favorable for growth (Van der Zaan and Van Loon, 1987). It is linked to seed-tuber physiological age and thus tuber age has considerable significance to commercial production through determining the speed and degree of stand establishment and ultimately crop yield.

Following harvest, seed-tubers pass through a number of developmental phases which are dependent upon their physiological age. Freshly- harvested tubers are initially dormant and unable to sprout even under conducive environmental conditions. Dormancy may last several months depending upon the cultivar and storage temperature. The dormant period is followed by the single-sprout stage where an initially slow-growing apical sprout exerts a high degree of apical dominance, inhibiting development of additional sprouts. Further aging results in loss of apical dominance, leading to multiple sprout production; eventually, the multiple sprouts begin to branch. Small-tuber development marks the final stage and is characterized by a loss in ability of the meristems to produce shoot growth (Krijthe, 1962). For the Russet Burbank cultivar the small-tuber formation stage would occur after approximately 3 years of storage at 4°C and 95% RH. In general, there is an "optimum" age where growth vigor is at a maximum, before which vigor increases as tubers are released from dormancy and after which vigor decreases as tubers advance in age and senesce.

The effect of seed-tuber age on growth vigor has been categorized by Krijthe (1962), Wurr (1978), Kawakami (1980) and Bodlaender and Marinus, (1987). The common view is that plants grown from physiologically older seed-tubers emerge more rapidly, develop more stems, initiate tubers earlier and at a lower leaf area index, and senesce earlier than plants from physiologically younger seed-tubers (Wurr, 1978; Bodlaender and Marinus, 1987). Also, final tuber yield is potentially reduced when older seed-tubers are planted (Wurr,

[1]The carbohydrate portion of this chapter is published in the *Ann Bot* (1989) 63 311-320.

1978), although the effect on yield depends on an interaction between age and length of the growing season (Iritani and Thornton, 1984).

Despite numerous reports characterizing the effects of seed-tuber age on plant growth and yield, few studies aimed at clarifying the physiological mechanisms underlying age-reduced growth potential have been attempted. Seed-tubers which have been aged over the long term (beyond the "optimum") are ideal for such studies because of clear-cut and consistent effects on plant growth and development, with which physiological differences can be correlated. The following reports illustrate the physiological complexity of the aging phenomenon.

Van Es and Hartmans (1987) have correlated increased peroxidase activity in sprouts with decreased sprouting capacity and decreased incubation period (time between the onset of sprouting of a seed-tuber and tuber formation on the new sprouts growing at 15-20°C in the dark). Following the period of maximum sprouting capacity, both vigor and incubation period are inversely related to storage time (chronological age). They reason that the ability to increase peroxidase activity confers a protective mechanism by which developing sprouts may offset the detrimental effects of age-dependent increases in free-radical formation. However, when seed-tubers age beyond the "optimum" there is a gradual reduction in sprout vigor which increasing peroxidase levels obviously do not counteract. Although peroxidase activity may be a good indicator of the physiological age of tubers which have passed their maximum sprouting capacity (Van Es and Hartmans, 1987), the increase in activity is a result rather than a cause of the effects of aging on growth and development.

It has also been speculated that, since apical dominance is lost with age, inability to translocate auxin or lower auxin levels in sprouts from older tubers may be responsible for reduced vigor (Knowles et al., 1985). However, treating aged tubers with auxin only partially restored growth and yield potential, suggesting additional age-related dysfunctions in metabolism.

Developing sprouts rely on seed-tuber reserves during the early stages of growth. Moorby (1978) states that the phloem of the mother tuber remains functional in the transfer of reserves for some time after the haulm has become autotrophic, and probably until reserves have been completely depleted or until decay is advanced. In a study comparing 5 and 17-month-old seed-tubers, rates of mobilization of seed-tuber nitrogen during sprout development were found to be dependent upon seed-tuber age (Knowles, 1987). Seventeen-month-old single-

eye seedcores produced 7 shoots per eye compared with a single shoot from 5-month-old cores, yet individual shoots from older cores had 4.6-fold less nitrogen than that from younger cores after 25 d of growth Decreased sink strength, along with increased competition among multiple shoots for tuber nitrogen reserves, probably contributed to loss of vigor on an individual-shoot basis (Knowles, 1987).

Age-related differences in apical dominance and mobilization of tuber nitrogen reserves do not entirely account for the loss of growth potential with advancing tuber age. Carbohydrates make up approximately 85% of tuber dry weight (Davies, 1984) and, by providing substrate for respiration and carbon skeletons for growth, are important to the plant-establishment phase of development. This study was initiated in order to investigate further the role of substrate-mobilization efficiency in the loss of growth potential from aged seed-tubers. Specifically, the efficiencies of mobilization and translocation of seed-tuber carbohydrate and nitrogen reserves during sprout development from different ages of seed-tubers were compared.

Materials and Methods

Potato (*Solanum tuberosum* cv. Russet Burbank) seed-tubers (PVX tested, Elite III) were stored at 4°C (95% RH) for 7 and 19 months. Prior to sampling, tubers were acclimated to room temperature for 24 hours in the dark and blocked for size. Single-eye main cores (1.8 cm diameter) were cut with a cork borer from the middle portion of the tubers perpendicular to the long (apical to basal) axis as previously described (Knowles, 1987). A 2 cm long core was cut from each end of the main core, so that one contained a single eye (seedcore) and the other only periderm (residual core). Apical and basal cores were avoided. Core fresh weight was recorded, seedcores were planted three per pot and the corresponding three residual cores were sliced into thin sections and lyophilized. Per cent dry weight of the residual cores was used to calculate zero-time (at time of planting) dry weight of the corresponding seedcores.

Seedcores were planted into 15-cm diameter pots containing a sterile, inert medium of peat:vermiculite (1:2, v/v). The pots were placed in a growth chamber with 25/18°C day/night temperature in a randomized complete block design with 4 blocks and 8 treatments (2 seed-tuber ages x 4 harvest dates). A combination of cool white fluorescent and incandescent bulbs provided 450 $\mu E\ m^{-2}\ s^{-1}$ light intensity for 16 hours per day. Pots were watered as needed and plants were

plants were harvested 12, 16, 20 and 24 days after planting. At each harvest, plants were divided into roots, shoots and seedcores and each yield component was frozen at -20°C and lyophilized. Shoot number and dry weight, along with root dry weight, were recorded, and the root/shoot ratio was calculated.

Lyophilized seedcores from each treatment were collectively ground into a fine powder with mortar and pestle and 50 mg of tissue was extracted in 10 ml cold (4°C) phosphate buffer (20 mM, pH 7.2) using a glass tissue grinder. The crude homogenate was centrifuged (1640 *g*, 10 min, 4°C) and the cold supernatant was analyzed for carbohydrates. Reducing sugars were assayed colorimetrically by the methods of Nelson (1944) and Somogyi (1952) using a 100 µl aliquot of the supernatant. Soluble carbohydrates were determined on a 50 µl aliquot of supernatant with the phenol-sulfuric acid reagent (Dubois et al., 1956). Both reducing sugars and total soluble carbohydrates were quantified on the basis of a glucose standard. Non-reducing sugar content was estimated by subtraction of reducing sugars from total soluble carbohydrates.

Starch content of the seedcores was determined by extracting 30 mg lyophilized tissue for 20 min in 4 ml boiling 80% (v/v) ethanol. The ethanol was decanted after centrifugation (1640 *g*, 15 min) and the extraction repeated. The supernatants were discarded, the pellet was resuspended in 4 ml distilled water and the starch was gelatinized for 1 hour at 100°C. Samples (50 µl) were then incubated with amyloglucosidase (E.C. 3.2.1.3, Sigma Chemical Co., St. Louis, MO) in 0.2 M acetate buffer (pH 4.7) for 1 hour at 55°C (Davies and Ross, 1984). The glucose released from the starch via the reaction with amyloglucosidase was quantified with glucose oxidase (Lloyd and Whelan, 1969). Carbohydrate content of the seedcores at zero-time was taken as the amount present in the corresponding residual cores averaged over all harvest dates.

For analysis of N, 100 mg of seedcore or shoot tissue was extracted (mortar and pestle) in cold (4°C) phosphate buffer (20 mM, pH 7.2) containing 0.1% (w/v) sodium bisulfite using a ratio of 1 ml buffer per 5 mg tissue. The crude homogenate was centrifuged (20,000 x *g*, 20 min, 4°C) and free amino N and soluble protein-N were determined on 100 and 400 µl of the supernatant, respectively. Ninhydrin was used to assay free amino N (Rosen, 1956) based on a leucine (10.7% N) standard. Soluble protein N was determined using a BSA (15.6% N) standard by a modification of the Lowry procedure (Bensadoun and Weinstein, 1976). Total N was determined on 30 mg of root, shoot or seedcore tissue by micro-Kjeldahl analysis (Black, 1972). Insoluble N was obtained by

subtraction of the soluble components from the total-N value. The amount of N contained within the seedcores at zero-time was taken as the amount of N in the corresponding residual cores averaged over all the harvest dates.

Plant growth, carbohydrate and N data were subjected to analyses of variance and, where appropriate, sums of squares were partitioned into individual degree-of-freedom components of both main effects and interactions. Based on the results of the analyses of variance, regression analysis was used to fit curves to the data.

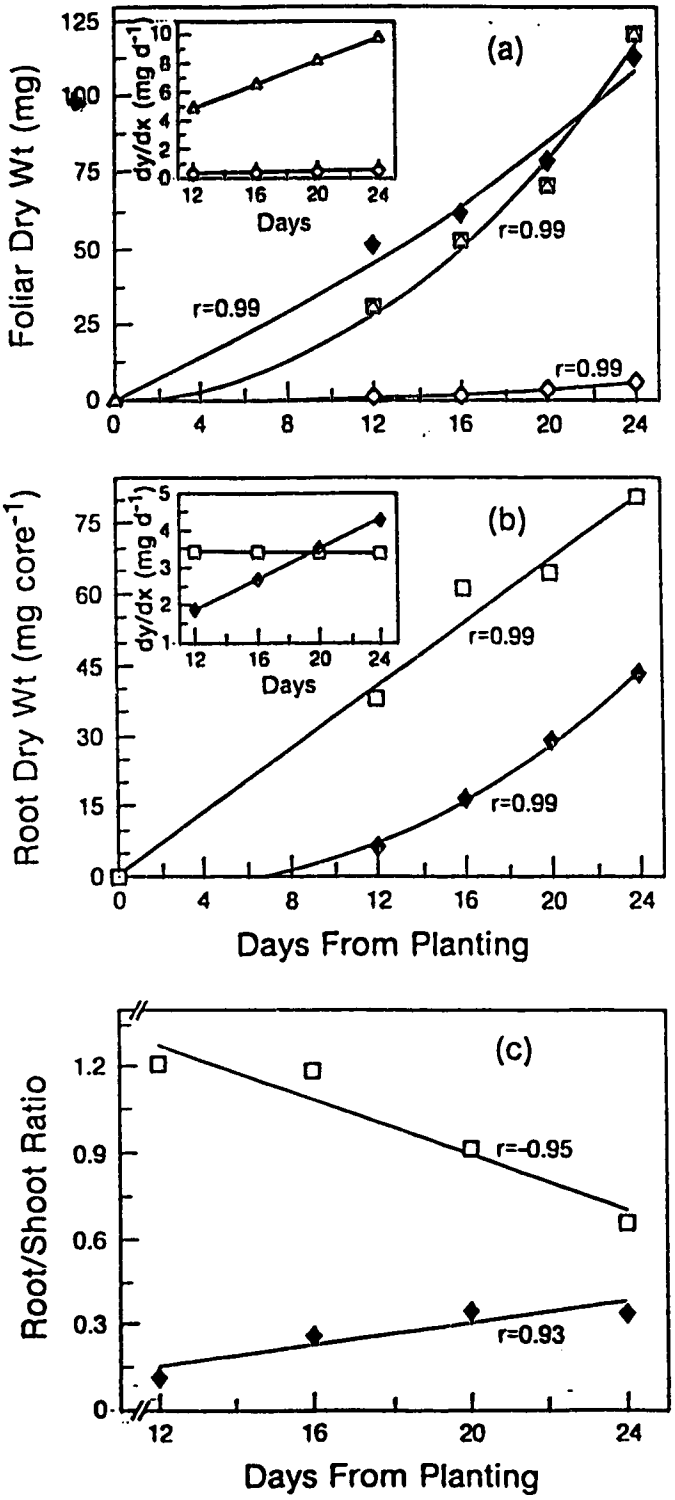
Results

Plant Establishment

The effect of tuber age on plant establishment is presented in Figure 1. Total shoot dry matter (mg /core) was produced at the same rate from 7 and 19-month-old seedcores throughout the study and no main effect of age was apparent (Fig. 1a). However, on a per-shoot basis, dry matter accumulation was 93% lower in plants growing from older seedcores than from younger seedcores after 24 d, reflecting a significant loss of apical dominance with age. On average, 16 shoots were produced from older seedcores and only 1 was produced from younger seedcores.

Root growth of plants developing from 7-month-old seedcores was linear and from 19-month-old seedcores was quadratic over the 24 day study period (Fig. 1b). After 24 d, 7-month-old seedcores had produced 45% more root dry matter than had 19-month-old seedcores. Seedcore age thus significantly influenced the partitioning of plant dry matter as illustrated by the root/shoot ratios depicted in Figure 1c. In general, the change in root/shoot ratio was linear with time for both seedcore ages; however, a highly significant age x time interaction was apparent. In younger seedcores, roots were produced at a faster rate than shoots during the initial stages of establishment, resulting in a root/shoot ratio greater than 1. In contrast, the rate of shoot growth from 19-month-old seedcores exceeded the rate of root growth throughout the study, as illustrated by a root/shoot ratio consistently less than unity. Furthermore, the root/shoot ratio of plants growing from younger cores decreased while that from older cores increased over the 24 day growth interval.

Figure 1. Time course of the accumulation of shoot dry weight (a), root dry weight (b), and root:shoot dry weight ratio (c), from single-eye cores from 7 (□, mg/core;△, mg/shoot) and 19-month-old (♦, mg/core;◊, mg/shoot) seed-tubers. (a) On a mg/shoot basis, F-value for the interaction of seedcore age x time (quadratic) was significant at $P < 0.01$ ($Y_{\triangle} = 0.78 - 0.22X + 0.21X^2$; $Y_{\diamond} = 0.08 - 0.06X + 0.02X^2$). On a mg/core basis, F-value for the main effect of time (quadratic) was significant at $P < 0.01$ ($Y_{\square} = 0.78 - 0.22X + 0.21X^2$; $Y_{\blacklozenge} = 1.06 + 2.95X + 0.07X^2$). Inset: Time course of shoot growth rate (mg dry wt/d). (b) F-values for the interactions of seedcore age x time linear and quadratic were significant at $P < 0.01$ and 0.05 levels, respectively ($Y_{\square} = 0.31 + 3.42X$; $Y_{\blacklozenge} = -0.12 - 0.59X + 0.10X^2$). Inset: Time course of root growth rate (mg dry wt/d). (c) F-value for the interaction of seedcore age x time (linear) was significant at $P < 0.01$ ($Y_{\square} = 1.84 - 0.05X$; $Y_{\blacklozenge} = -0.09 + 0.02X$).


(a)
Foliar Dry Wt (mg)
dy/dx (mg d-1)
Days
r=0.99
r=0.99
r=0.99
(b)
Root Dry Wt (mg core-1)
dy/dx (mg d-1)
Days
r=0.99
r=0.99
Days From Planting
(c)
Root/Shoot Ratio
r=-0.95
r=0.93
Days From Planting

Plant (roots + shoots) dry matter accumulation from the two ages of seedcores was linear and there was an interaction with age (Fig. 2a). Plant growth rate from 7-month-old seedcores was 24% higher than that from 19-month-old seedcores (8.1 versus 6.5 mg /d).

.Mobilization of Seedcore Dry Matter

Mobilization of seedcore dry matter was also linear and occurred at the same rate (approx. 17.7 mg dry weight loss per day) for both 7 and 19-month-old seedcores (Fig. 2b). The main effect of age was highly significant. At planting, older seedcores contained 15% less dry matter than younger seedcores and this difference was maintained throughout the study.

The relationship between seedcore dry weight (% original) and plant dry weight (mg/core) was inverse and linear for both ages of seedcores (Fig. 2c). On a per-core basis, 19-month-old seedcores lost 0.25% (approx. 2.8 mg) of their original dry matter for every mg increase in plant dry weight, compared with only 0.15% (2.1 mg) from 7-month-old seedcores.

Mobilization of Seedcore Carbohydrates

Mobilization of seedcore carbohydrates as a function of time is shown in Figure 3. Over the 24 day growth interval, there was a linear decrease in the total amount and concentration of starch within both ages of seedcores (Fig. 3a) . Furthermore, seedcore age had no effect on the rate of starch mobilization which averaged 17 mg /core /d (or 14.6 mg/ g dry weight/d). Disregarding the cellulose content, starch accounted for 86 and 82% of the zero time carbohydrate pool in 7 and 19-month-old seedcores, respectively. Older cores contained 35% less starch on a per-core basis (20% less on a mg /g dry weight basis) at planting.

Despite a relatively high level of variation, total soluble carbohydrates increased linearly from 132 to 190 mg/ core in both seedcore ages (Fig. 3b, inset). On a concentration basis, soluble carbohydrates increased at an average rate of 4.5 mg /g dry weight/d in both core ages; however, older seedcores had 20% more than younger seedcores for the duration of the study (Fig. 3b).

Figure 3c (inset) illustrates the change in reducing sugar content (mg/core) with time. A highly significant age x time (quadratic) interaction was evident. Compared with 7 month old seedcores, 19 month old seedcores contained 38% more reducing sugars at planting. Reducing sugars increased in

Figure 2. Time course of the accumulation of plant (roots + shoots) dry weight (a), and loss in seedcore dry weight (b), from single-eye cores from 7 (□) and 19-month-old (♦) seed-tubers. (a) F-value for the interaction of age x time (linear) was significant at $P < 0.05$ ($Y_{□} = 8.1\ X - 11.1$; $Y_{♦} = 6.5X - 10.5$). (b) F-values for the main effects of seedcore age and time (linear) were significant at $P < 0.01$ ($Y_{□} = 1.34 - 0.017X$; $Y_{♦} = 1.14 - 0.018X$). (c) Loss of seedcore dry matter with increasing plant dry weight from 7 and 19-month-old seedcores. Curves presented were derived from the calculated Y-values from (a) and (b) ($Y_{□} = 94.9 - 0.15X$; $Y_{♦} = 97.7 - 0.25X$).

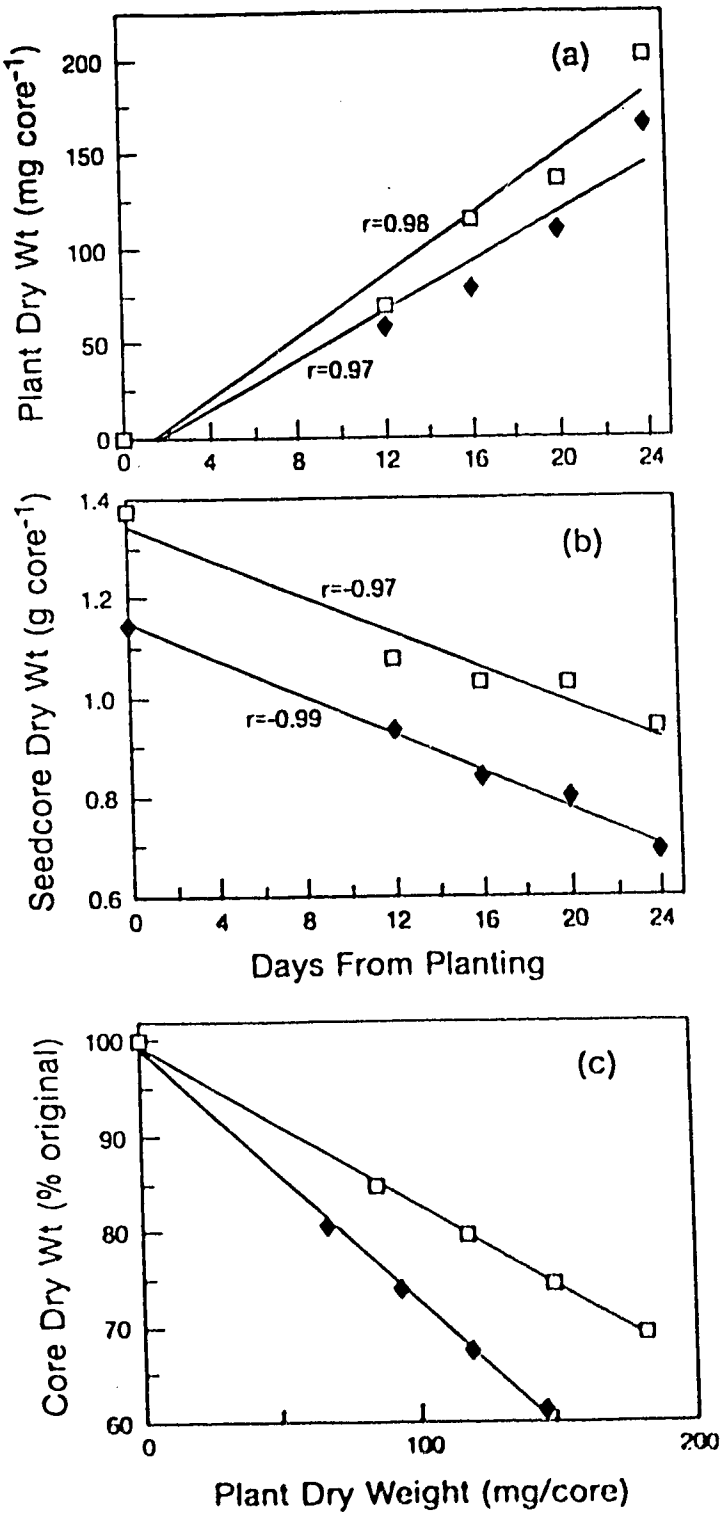
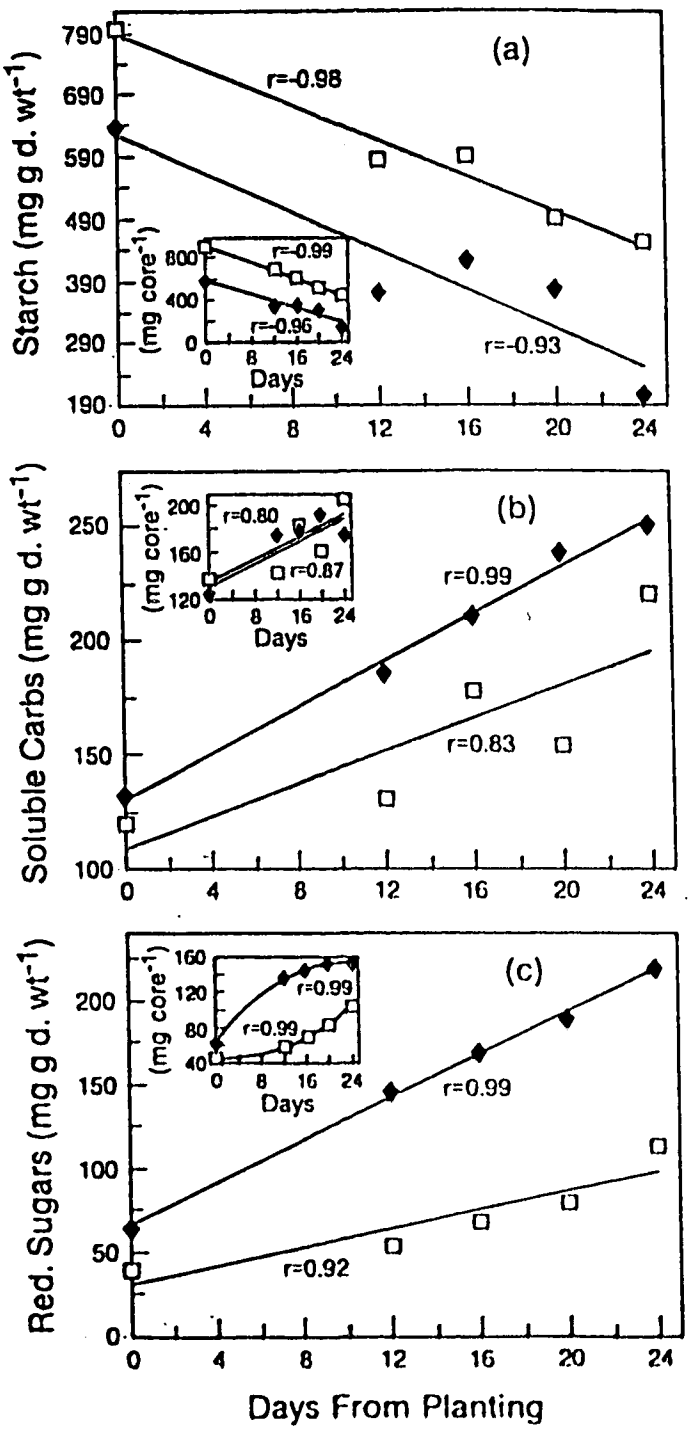

(a)
Plant Dry Wt (mg core^{-1})
200
150
100
50
0
r=0.98
r=0.97
0
4
8
12
16
20
24
(b)
Seedcore Dry Wt (g core^{-1})
1.4
1.2
1.0
0.8
0.6
r=-0.97
r=-0.99
Days From Planting
(c)
Core Dry Wt (% original)
100
90
80
70
60
0
100
200
Plant Dry Weight (mg/core)

Figure 3. Time course of starch degradation (a), increase in soluble carbohydrates (b), and reducing sugars (c), within single-eye cores from 7 (□) and 19-month-old (♦) seed-tubers. (a) F-values for seedcore age and time (linear) main effects were significant at $P < 0.01$ ($Y_{□} = 790 - 13.9X$; $Y_{♦} = 629 - 15.3X$). Inset: F-values for the main effects of seedcore age and time were significant at $P < 0.01$. (b) F-values for seedcore age and time (linear) main effects were significant at $P < 0.01$ ($Y_{□} = 108 + 3.7X$; $Y_{♦} = 130 + 5.2X$). Inset: F-values for the main effect of time (linear) was significant at $P < 0.01$. (c) F-value for the seedcore age x time (linear) interaction was significant at $P < 0.05$ ($Y_{□} = 31 + 2.8X$; $Y_{♦} = 65 + 6.4X$). Inset: F-value for the interaction of seedcore age x time (quadratic) was significant at $P < 0.01$.


(a)
Starch (mg g d. wt^{-1})
790
690
590
490
390
290
190
r=-0.98
r=-0.93
(mg core^{-1})
800
400
0
r=-0.99
r=-0.96
0 8 16 24
Days
0 4 8 12 16 20 24
(b)
Soluble Carbs (mg g d. wt^{-1})
250
200
150
100
r=0.99
r=0.83
(mg core^{-1})
200
160
120
r=0.80
r=0.87
0 8 16 24
Days
0 4 8 12 16 20 24
(c)
Red. Sugars (mg g d. wt^{-1})
200
150
100
50
0
r=0.99
r=0.92
(mg core^{-1})
160
120
80
40
r=0.99
r=0.99
0 8 16 24
Days
0 4 8 12 16 20 24
Days From Planting

both ages of cores; however, from 12 to 24 days the rate (mg /core/d) of increase in 19-month-old cores gradually decreased whereas it increased in 7-month-old cores. On a concentration basis, reducing-sugar content of 7-month-old seedcores was 53% lower than that of 19-month-old seedcores at planting, but the rate of increase was 2.3-fold higher in the older cores. By the end of the growth period, reducing-sugar concentration within younger cores had increased 3.7-fold to 114 mg/g dry weight In older seedcores, the 3.4-fold increase resulted in a final concentration of 220 mg/g dry weight.

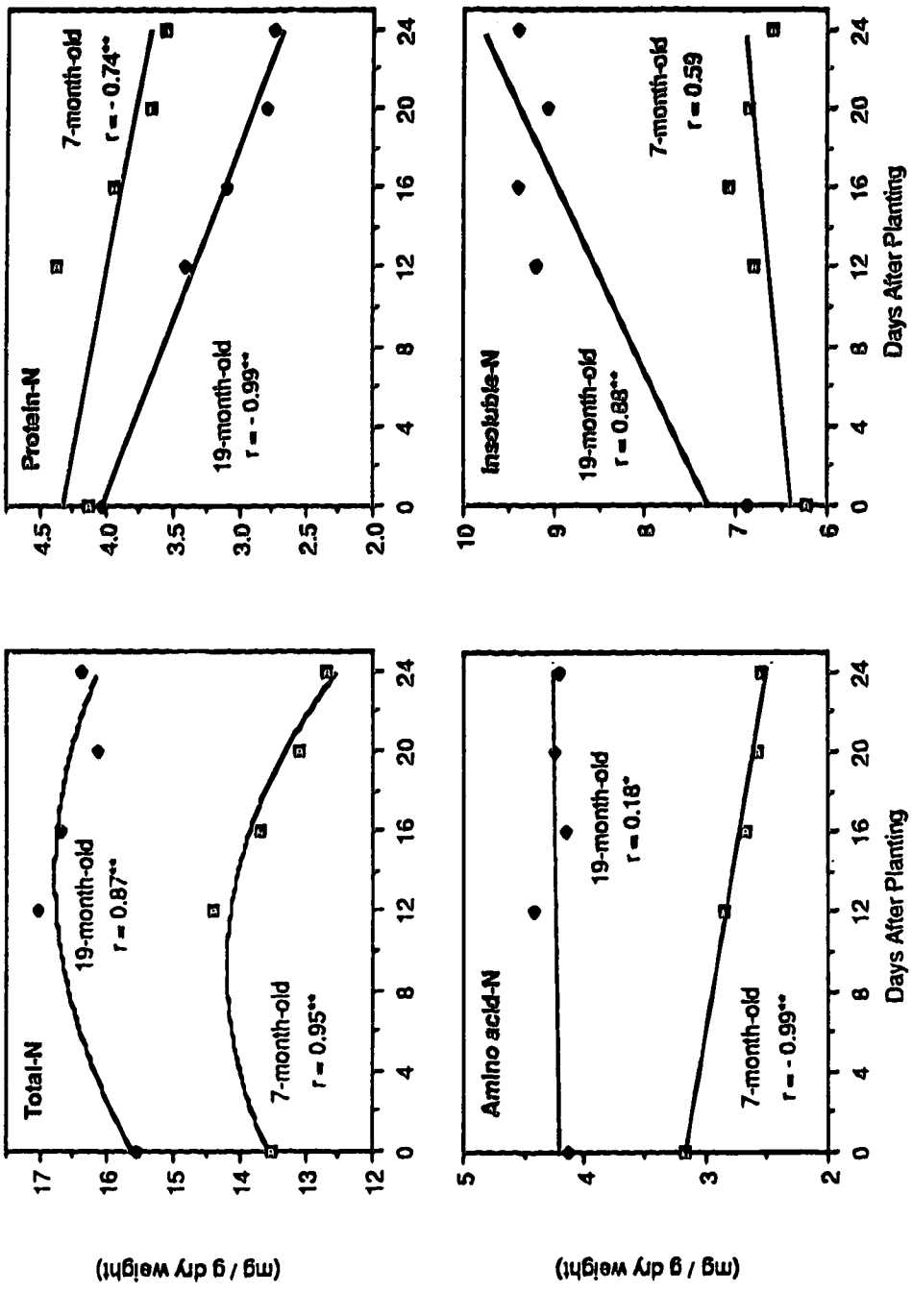
Although non-reducing sugars (mainly sucrose) fluctuated over the growth period in both ages of seedcores, a significant age effect was characterized (data not shown). On average, the concentration remained constant in 7-month-old seedcores at 91 mg/g dry weight. In 19-month-old seedcores, the initial concentration was 69 mg/g dry weight, but this declined quadratically ($r = -0.86$, $P < 0.05$) to 33 mg/g dry weight by day 24.

Mobilization of Seedcore Nitrogen

The influence of seed-tuber age on mobilization and translocation of seed-tuber N reserves during sprouting was studied in conjunction with carbohydrate metabolism. Total-N of seedcores from both tuber ages averaged 16.1 mg/core at planting, and seed-tuber age had no effect on the rate of total-N loss during sprouting (results not shown). Over the sprouting interval, total-N was translocated at an average rate of 0.14 mg/core·d, and by day 24, an average of 3.5 mg/core (22 % of total-N) was lost from both young and old seedcores. Compared with 19-month-old cores, seedcores from 7-month-old tubers contained 26 % more soluble protein-N per core and 12 % less free amino-N per core at planting, and these differences were maintained over the 24 d growth period. In both 7 and 19-month-old seedcores, average rates of soluble protein and free-amino-N loss were 0.07 mg/core/d and 0.04 mg/core/d, respectively. Insoluble-N content was not affected by seedcore age and changed quadratically with sprouting. Insoluble-N increased 8% to 8 mg/core by 12 DAP, then decreased to 6.7 mg/core by day 24.

The change in seedcore-N concentration with time is shown in Figure 4. In general, concentrations of total, free amino and insoluble-N were higher, and the concentration of soluble protein-N was lower, in older compared with younger cores. Independent of tuber age, the concentration of total-N changed quadratically with sprouting, and 19-month-old seedcores contained an

Figure 4. Change in N reserves (mg/g dry wt) of single-eye seedcores from 7 (□) and 19-month-old (◆) potato seed tubers over a 24 d sprouting interval. F-values for the age x time (linear) interactions were significant at the 0.05, 0.05 and 0.01 levels of soluble protein, free amino and insoluble-N, respectively. The trend in total-N was quadratic and the F-value for the main effect of age was significant at the 0.01 level.


Total-N
19-month-old
r = 0.87**
7-month-old
r = 0.95**
Protein-N
7-month-old
r = - 0.74**
19-month-old
r = - 0.99**
Amino acid-N
19-month-old
r = 0.18*
7-month-old
r = - 0.99**
Insoluble-N
19-month-old
r = 0.88**
7-month-old
r = 0.59
(mg / g dry weight)
(mg / g dry weight)
Days After Planting
Days After Planting

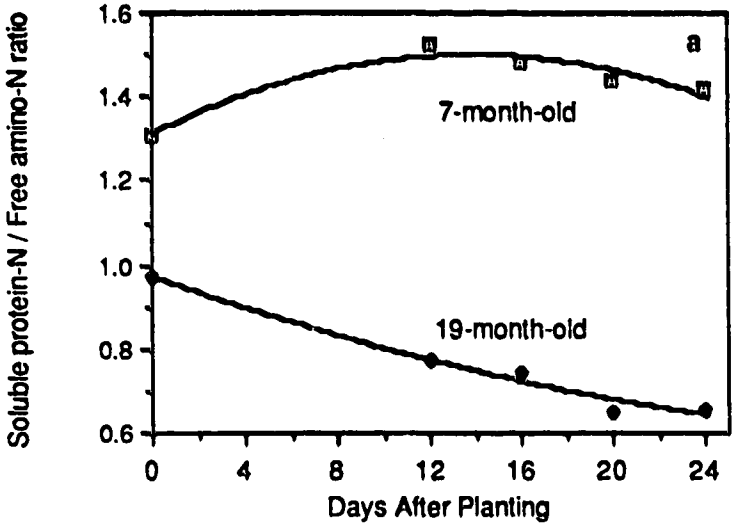
average of 21 % more total-N than 7-month-old cores throughout the study. A significant age x time interaction characterized a linear decrease in soluble protein-N concentration. Soluble protein-N of older cores decreased at a rate 2-fold faster than in younger cores and by day 24, older cores had lost 1.4 mg/g dry weight soluble protein-N compared with 0.7 mg/g dry weight from younger cores. Free amino-N decreased linearly in younger cores to 2.5 mg/g dry weight by day 24, a 22 % reduction from the time of planting. Concentration of free amino-N in older cores, however, remained constant over the sprouting interval, averaging 4.2 mg/g dry weight. Although insoluble-N concentration was 14 % higher in the older cores at planting, it increased 5-fold faster than in the younger cores reaching 9.7 mg/g dry weight by day 24, 41 % greater than in younger cores at that time.

Figure 5 illustrates the distribution of N (mg/g dry weight) among the soluble protein, free amino and insoluble-N pools within the 7 and 19-month-old seedcores over the 24-d interval. The trend in the ratio of seedcore soluble protein/free amino-N was quadratic and a significant age x time interaction was evident (Fig. 5a). In older cores, this ratio was consistently less than unity indicating that free amino-N concentration exceeded that of soluble protein throughout the study. Also, this ratio decreased with time in 19-month-old seedcore tissue from 0.97 to 0.63. In younger seedcores, the reverse situation occurred. The concentration of soluble protein-N was greater than that of free amino-N, and the resulting ratio was greater than 1 at all times. In 7-month-old seedcores, the ratio between these N pools increased to 1.46 by day 24, a level 2.3-fold greater than that of older cores.

Seedcore soluble protein/insoluble-N ratio (Fig. 5b) decreased linearly for both seedcore ages, but at a rate 1.7-fold faster in older cores than in younger cores. At planting, this ratio was 18 % greater in 7 month-old compared with 19-month-old cores; however, by 24 DAP the ratio was 62 % higher in cores from the younger seed-tubers.

The relationship between total seedcore N and shoot dry weight was inverse and linear (Fig. 6a). Over the sprouting interval, for each mg shoot dry matter gained, both 7 and 19-month-old seedcores lost total N at the same rate. By day 24, both seedcore ages had lost an average of 3.5 mg N/core and shoots had gained an average of 118 mg dry matter.

Figure 5. Time course of (a) soluble protein/free amino-N (mg/g dry wt) and (b) soluble protein/insoluble-N (mg/g dry wt) ratios for single-eye seedcores from 7 (□) and 19-month-old (◆) potato seed-tubers. *,**F-values for the age x time (quadratic, a; linear, b) were significant at the 0.01 level.


Soluble protein-N / Free amino-N ratio
1.6
1.4
1.2
1.0
0.8
0.6
a
7-month-old
19-month-old
0
4
8
12
16
20
24
Days After Planting

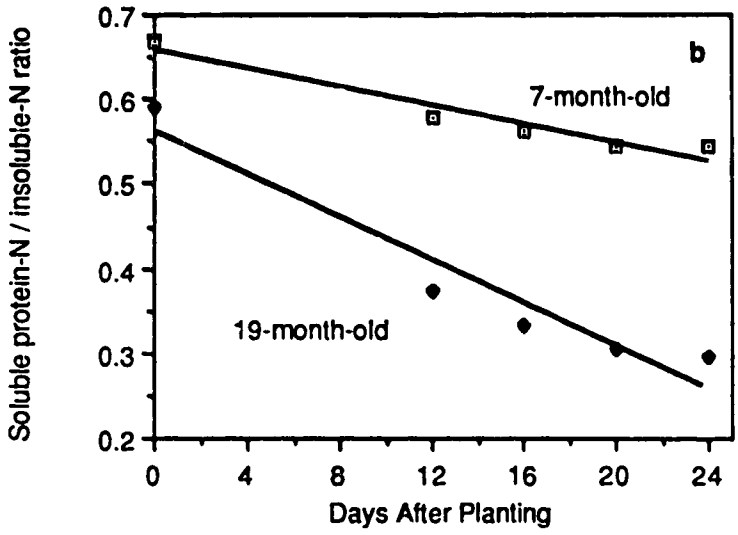
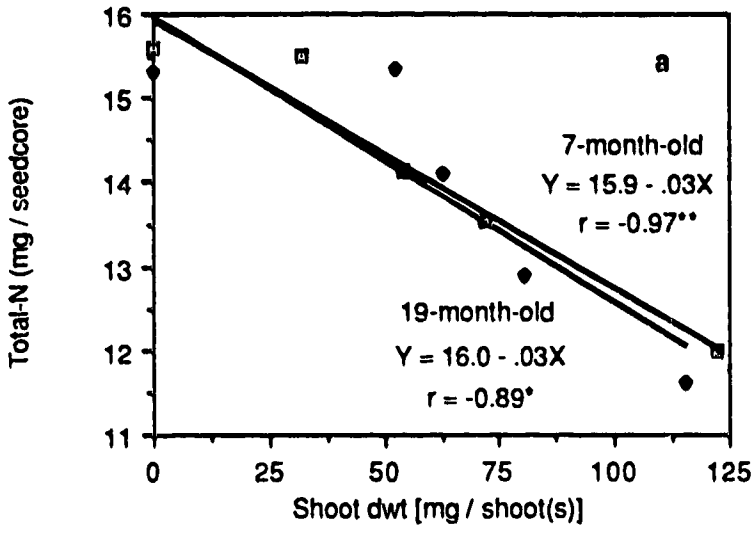
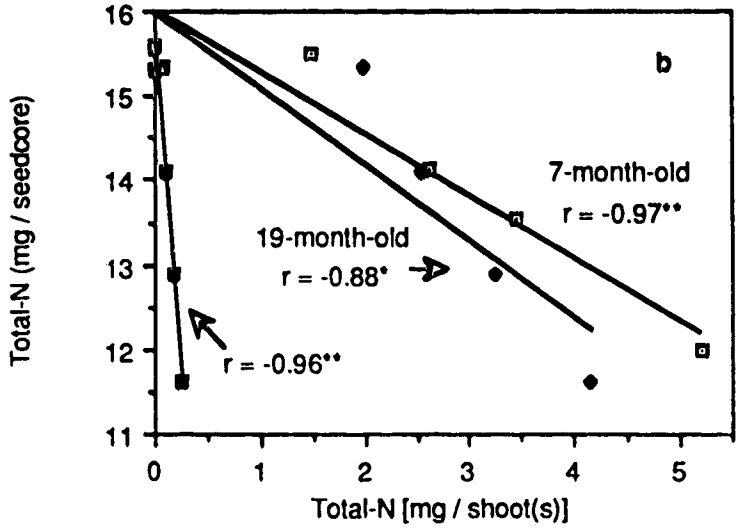

Soluble protein-N / insoluble-N ratio
0.7
0.6
0.5
0.4
0.3
0.2
b
7-month-old
19-month-old
0
4
8
12
16
20
24
Days After Planting

Figure 6. (a) Loss of total seedcore-N with increasing shoot dry weight during sprouting of single-eye seedcores from 7 (□) and 19-month-old (◆) potato seed-tubers. *,**F-values for the linear regressions were significant at the 0.05 and 0.01 levels, respectively. (b) Loss of total seedcore-N with increasing total-N in shoots developing from single-eye seedcores from 7 (□) and 19-month-old (◆,mg /shoots:■,mg/shoot) potato seed-tubers. *,**F-values for the linear regressions were significant at the 0.05 and 0.01 levels, respectively.


a
7-month-old
Y = 15.9 - .03X
r = -0.97**
19-month-old
Y = 16.0 - .03X
r = -0.89*
Total-N (mg / seedcore)
Shoot dwt [mg / shoot(s)]
16
15
14
13
12
11
0
25
50
75
100
125


b
7-month-old
r = -0.97**
19-month-old
r = -0.88*
r = -0.96**
Total-N (mg / seedcore)
Total-N [mg / shoot(s)]
16
15
14
13
12
11
0
1
2
3
4
5

Seedcore-N was also inversely related to total-N of shoots (Fig. 6b). Per mg total-N gained by the shoots, 19-month-old seedcores lost total-N 23 % faster than 7-month-old cores. Yet, by day 24, total-N of shoots from older cores was significantly less than that from younger cores. In this study, older seedcores produced an average of 16 shoots, while 7-month-old seedcores produced only one. On a single shoot basis (Fig. 6b), each shoot from older cores accumulated 95% less total-N than that from younger cores by the end of the study. Also, for each mg total-N gained per shoot, older cores lost 16.2 mg total-N.

Change in N (mg/shoot(s) and mg/g dry weight) content of shoots growing from 7 and 19-month-old seedcores is shown in Table 1. The rate of accumulation of total-N (mg/shoot(s)) depended upon seedcore age. Total-N of shoots from both ages of seedcores increased linearly with time; however, the rate of increase was 1.6-fold greater in shoots from younger cores compared with those from older cores. Seed-tuber age had no effect on content or rate of increase in soluble protein or insoluble shoot N. Soluble protein and insoluble-N averaged 1.5 mg and 2.8 mg, respectively in shoots from the 7 and 19-month-old seedcores by day 24. A significant age x time (linear) interaction characterized the accumulation of free amino-N (mg/shoot(s)) resulting in 30% more free amino-N in shoots from younger seedcores compared with those from older seedcores by the end of the study.

The concentration of total-N changed quadratically in shoots from both ages of seedcores (Table 1). A significant age effect was apparent, and over the growth interval, shoots from older cores averaged 20% less total-N (mg/g dry weight) compared with those from younger cores. Concentration of soluble protein-N was similar for shoots from both ages of seedcores, averaging 12.4 mg/g dry weight throughout the study. Both free amino and insoluble-N decreased over the 24 d sprouting interval and the concentration of these N-fractions were significantly higher in shoots from the younger cores compared with those from the older cores.

Discussion

Even though the collective dry weight of the multiple shoots from older seedcores was equivalent to that of the single shoot from younger seedcores throughout the study (Fig. 1a), age-related differences between plant (root + shoots) total dry matter and rate of accumulation were evident (Fig. 2a). These

Table 1: Nitrogen (mg/shoot(s) and mg/ g dry weight) of shoots produced per single-eye seedcore from potato seed-tubers stored 7 and 19 months.

Age (months)	Harvest Date (HD)	mg / shoot(s)				mg /g dry weight			
		total	soluble protein	amino acid	insoluble	total	soluble protein	amino acid	insoluble
7	12	1.49	-	-	-	46.74	-	-	-
	16	2.60	0.65	0.32	1.63	48.40	11.72	6.25	30.43
	20	3.44	0.89	0.37	2.18	48.23	13.39	5.21	30.63
	24	5.19	1.56	0.43	3.20	42.04	12.72	3.59	25.73
19	12	1.98	-	-	-	37.40	-	-	-
	16	2.54	0.73	0.29	1.52	40.51	11.78	4.72	24.01
	20	3.23	1.03	0.28	1.92	40.28	12.74	3.74	23.80
	24	4.14	1.39	0.32	2.43	36.56	11.99	2.78	21.79
Age (A)[a]		ns[b]	ns	0.01	ns	0.01	ns	0.01	0.01
Time $(T)_{LT}$		0.01	0.01	0.01	0.01	0.05	ns	0.01	0.01
T_{QT}		ns	ns	0.01	ns	0.01	ns	ns	ns
AXT_{LT}		0.05	ns	0.05	ns	ns	ns	ns	ns
AXT_{QT}		ns	ns	ns	ns	ns	ns	ns	ns

[a] Source of variation (LT and QT subscripts denote linear and quadratic trends, respectively). [b] Level of significance for indicated sources of variation (ns = not significant).

differences are attributed to slower establishment of roots on plants growing from 19-month-old cores (Fig. 1b). Root growth rate (mg/core/d) was constant with time for plants from younger seedcores; it increased linearly for plants growing from older seedcores (Fig. 1b, inset). In contrast to older cores, younger cores were able to initiate and develop roots very early in the sprouting period. Root/shoot ratios were thus influenced by seedcore age (Fig. 1c). These data illustrate that during the early stages of establishment, plants developing from older seedcores have a diminished capacity for root growth.

Single-eye seedcores from 19-month-old seed-tubers averaged 16 shoots, compared with only 1 shoot from 7-month-old seedcores, reflecting an age-dependent loss in apical dominance. On an individual basis, shoot dry weight from older cores was thus 16-fold lower than that from younger cores. These findings are in agreement with those reported by Bodlaender and Marinus (1987) and Knowles (1987). The characteristic loss in apical dominance is apparently associated with reduced levels of auxin in sprouts developing from tubers of advanced age. This is indirectly supported by the fact that apical dominance can be restored by treating older seedcores with auxin prior to planting (Knowles et al., 1985).

Reduced auxin levels, associated with loss of apical dominance from older seed-tubers, may play a role in the reduction of sprout vigor by influencing root growth. When potato tubers sprout, adventitious roots are produced from the lower nodes of the developing shoots. With advancing age, reduced ability of meristems to produce and/or translocate auxin may limit the capacity of shoots from older seedcores to initiate and develop roots as quickly as those from younger seedcores.

To test the effect of auxin on root development, 5 and 17-month-old seedcores were treated with 0 to 150 mg/l NAA and root growth was compared. After 28 days of growth, treatment of 17-month-old seedcores with 50 mg/l NAA increased root dry weight (mg/core) 23%, resulting in root growth equivalent to that produced from untreated 5-month-old seedcores. Increasing the level of NAA applied to 5-month-old seedcores resulted in a linear decrease in root dry matter. However, NAA did not fully restore shoot vigor to older cores (unpublished results). Similarly, earlier studies reported only partial recovery of shoot-growth potential from aged seed-tubers treated with NAA (Knowles, et al., 1985). Auxin concentration is known to regulate differentiation of roots in tissue culture and is commonly used as a rooting agent for propagating many plant

species. Thus, it appears that lack of auxin may be an important factor dictating reduced vigor of shoots from aged seed-tubers through limiting rooting potential during early establishment.

The per cent dry weight of seedcores at planting was dependent upon age of the seed-tuber. On average, younger cores contained 24% dry matter, while older cores were only 20% dry matter, an initial dry weight difference between the two ages of about 200 mg (Fig. 2b). The lower dry matter content of older seedcores is attributed to higher cumulative respiratory losses during the additional 12 months of storage. Evidence in support of this comes from a previous study which estimated that during storage (4°C, high humidity) tubers lose approximately 0.3% of their dry matter per month through respiration (Burton, 1978).

Despite age-related differences in seedcore dry matter at planting, rates of mobilization were the same, averaging 17.7 mg/core·d and resulting in 424 mg dry weight lost from both ages over the 24 day growth interval (Fig. 2b). Seedcore decay was not evident during the study and therefore the 35% weight loss was mainly due to respiration and translocation of reserves to developing plants.

Since plants were not photosynthetically active during the initial 12 days of growth, the absolute amount of seedcore dry weight mobilized to directly support plant development could be calculated and compared for both ages. The average dry weight loss was 212 mg during this period (Fig. 2b). Because of a significantly higher growth rate, plants developing from younger seedcores had accumulated 26% more dry matter (86 mg) than those from older seedcores (68 mg) after 12 days (Fig. 2a). Hence, in producing a significantly lower amount of plant dry matter, older seedcores lost 152 mg compared with 118 mg dry weight from younger seedcores. This reduced efficiency of reserve mobilization from older seedcores extended through the entire 24 day growth interval as illustrated in Figure 2c. For every mg increase in plant dry weight from younger seedcores, 2.1 mg of core dry matter was lost compared with 2.8 mg from older cores (a 33% difference). The increased rate of core dry weight loss is consistent with higher rates of respiration in older seed-tubers during the plant-establishment stage of development as previously characterized (Knowles, 1983).

Mobilization of Seedcore Carbohydrates

Total carbohydrates represented the bulk of seedcore dry matter (77 and 63% in 7 and 19-month-old seedcores, respectively) and, from a sheer quantitative standpoint, are unlikely to be limiting to sprout development.

However, as Davies (1984) points out, if the rates of mobilization to translocatable products and/or the rate of translocation to developing sprouts were somehow altered, then availability of tuber reserves may become a limiting factor to growth. The influence of tuber age on these rates was thus considered.

In this study, total carbohydrates decreased linearly and at the same rate (approx. 15 mg/core/d or 10 mg/g dry weight/d) in both ages of seedcores (results not shown). Movement of carbohydrate from shoots to the mother seed-tuber during plant establishment is negligible (Oparka and Davies, 1984). Therefore, the rate of translocation to shoots was not affected by seed-tuber age. This was somewhat surprising in light of the fact that 19-month-old seedcores produced 16 shoots compared with one from 7-month-old seedcores. Since the collective dry weight of the 16 shoots from 19-month-old cores was equivalent to that of the single shoot from 7-month-old cores throughout the study, the results would suggest that carbohydrate reserves, whether limited by rates of mobilization or translocation, are sufficient to support growth of only a single shoot. Apparently, the collective sink strength of the multiple shoots developing from older cores was equivalent to that of the single shoot developing from younger cores.

Differences in the rate of mobilization of carbohydrates to translocatable products are evident upon examination of the various pools. Starch is the major carbohydrate reserve, mobilized as hexose phosphate esters (Romberger and Norton, 1961); it is used to support respiration and sucrose synthesis. Sucrose is the major translocatable carbohydrate in potatoes (Avigad, 1982). The initial difference in starch content between 7 and 19-month-old seedcores (Fig. 3a), due to a combination of respiratory losses and breakdown over the extended storage period, more than accounts for the initial difference in seedcore dry weight (Fig. 2b).

The equilibrium between the various carbohydrate pools within a tuber depends upon its physiological age. In younger seed-tubers, the equilibrium favors starch (with reducing-sugar and sucrose content remaining at relatively low levels). As tubers age in storage, the equilibrium shifts, resulting in the accumulation of reducing sugars and sucrose. This phenomenon is termed senescent sweetening (Isherwood, 1976) and accounts for the higher content and concentration of reducing sugars in older cores at planting (Fig. 3c and inset).

The rate of starch breakdown was not influenced by tuber age (Fig. 3a). Similarly, seed-tuber age did not affect the rate of accumulation of soluble carbohydrates, consisting mainly of reducing sugars and sucrose (Fig. 3b).

However, the rate of accumulation of reducing sugars (mg/g dry weight) was 2.3-fold greater in older than in younger cores (Fig. 3c). This buildup would suggest that the synthesis of sucrose (which would then be translocated to developing sprouts) from glucose and fructose is less efficient in older cores; alternatively, the hydrolysis of sucrose is occurring at a faster rate than translocation. Both scenarios would account for an increasing concentration of reducing sugars within older seedcores during sprouting.

The level of sucrose remained constant in younger cores over the growth period, indicating efficient transport of carbohydrate to the developing sprout. Indeed, reducing sugars do not accumulate appreciably in younger cores until the end of the study (Fig. 3c). In contrast, sucrose declined steadily in older cores, concomitant with a rapid increase in reducing-sugar concentration ($r = -0.90$, $P < 0.05$), implying a less efficient transfer of available carbohydrate reserves to developing sprouts. Increased sucrose hydrolysis (possibly a function of an age-induced increase in invertase activity or level), coupled with reduced sink strength of single sprouts from aged tubers, may account for the large accumulation of reducing sugars in older cores and hence may contribute to the loss of sprout vigor on an individual basis.

In summary, it is apparent from the data that as seed-tuber age advances, apical dominance decreases and the strength of individual shoots as sinks for seedcore carbohydrates also decreases in proportion to the number of shoots produced. There was no direct evidence for age-reduced efficiency of translocation of carbohydrate from seedcore to shoots. However, the efficency of mobilization of seedcore carbohydrates to translocatable products was different in the two ages of seedcores. Reducing sugars accumulated in cores from older seed-tubers, apparently because of a greater rate of hydrolysis of sucrose. Sucrose and reducing sugar concentration within younger cores remained relatively constant. As was the case with seedpiece nitrogen (Knowles, 1987), reduced vigor of individual shoots during the establishment stage of plant growth from older seedcores is closely related to decreased sink strength and increased competition among multiple shoots for a limited supply of carbohydrate. The potential amount of seedpiece carbohydrate does not appear to be the limiting factor. Rather, the data suggest that a higher rate of sucrose hydrolysis within older cores is responsible for limiting the amount of carbohydrate translocated.

Mobilization of Seedcore Nitrogen Reserves

Nitrogen mobilization from 7 and 19-month-old seedcores was monitored over a 24-d interval of plant establishment. The objectives were to determine if the absolute amount of seedcore-N was limiting sprout growth; if increased competition for N between the multiple shoots of older seedcores limited the growth of individual shoots; or if the rates of mobilization and/or translocation of seedcore-N to shoots were influenced by seed-tuber age.

Total-N lost by older seedcores accounted for 91 % of that appearing in the shoots, while shoots from 7-month-old seedcores derived only 74 % of their total-N from the seedcore. The shoots from younger seedcores apparently obtained N from an external source. However, even in the presence of added nutrients, developing potato sprouts are highly dependent upon tuber reserves (Headford, 1962) and will continue to deplete tuber reserves for some time after becoming autotrophic (Moorby, 1978). Similarly, in developing cereal seedlings when external N is supplied, the major source of amino acids for the seedlings is hydrolysis of endosperm proteins (Oaks, 1983). Though seedcore-N reserves were evidently not the sole source of N for the shoots, the majority of shoot-N originated from the seedcore.

Shoots from younger cores contained 1.4 mg more total-N than was translocated from the seedcore compared with less than 1 mg more in those from older cores. The plants from younger cores thus appeared to be more efficient at taking up exogenous N than those from older cores. Dry matter of the root system produced by shoots from older seedcores was only 50 % of that from younger cores by day 24 (Fig. 1b). The results suggest that the ability of plants from older cores to accumulate N may be limited by age-induced effects on plant morphology, in particular, root production.

On an individual shoot basis, 16.2 mg of seedcore-N was translocated per mg N gain in the single shoots from older cores (Fig. 6b). Single shoots from 19-month-old seedcores therefore received a smaller portion of the total seedcore-N, reflecting a high degree of competition between the multiple shoots from the older seedcores.

Of the total-N translocated by the seedcores, individual shoots from 19-month-old seedcores received only 7 %, while the single shoot from younger cores received 100 %. Interestingly, vigor (dry matter/ shoot) of shoots from older cores was 93 % lower than that from younger cores after 24 d of growth (Fig. 1). The data suggest that much of the age-induced difference in shoot vigor may be

due to a limited supply of N. The multiple shoots from older cores are apparently competing for a fixed amount (approximately 4 mg) of translocatable seedcore N.

After 24 d of growth total shoot dry matter was equal from both ages of seedcores, and the total-N lost by the cores was also equal (Fig. 6a). However, total-N (mg/g dry weight) of the shoots from younger cores was significantly higher than that from older cores (Table 1). Due to the presence of external source of N for growth (ie. non-seedcore derived N), it is difficult to equate differences in shoot-N directly to age-related differences in sink strength. Nevertheless, it is apparent that collectively, the 16 shoots produced by each 19-month-old seedcore were a weaker sink for N compared with the single shoot from each 7-month-old core. Furthermore, on a single shoot basis, each shoot from younger cores, was a much stronger sink for N than that from older cores.

Since both ages of seedcores initially contained and translocated equal amounts of total-N, the limiting factor in shoot-N accumulation was not the absolute amount of seedcore-N. Also, translocation from source to sink (seedcore to shoots) was apparently not affected by tuber age since both ages of seedcores lost N at the same rate.

Mobilization of N reserves to translocatable forms was dependent upon seedcore age, as shown by the change in concentration of the various N pools over time (Fig.4). During sprouting, seedcore protein may be lost by hydrolysis to free amino acids which are then translocated to the shoots, or by conversion to insoluble-N which remains relatively immobile (eg. through denaturation). In younger cores, both soluble protein and free amino-N decreased at a rate of 0.03 mg/g dry weight/d, while insoluble-N remained constant over the 24-d interval. The slow decline in the soluble protein/insoluble-N ratio (Fig. 5b) suggests that a large proportion of the protein is mobilized and translocated to the developing shoots and little is insolubilized during sprouting of the 7-month-old seedcores. In older seedcores, protein-N was lost at a rate of 0.06 mg/g dry weight·d, 2-fold faster than in younger cores (Fig. 4). However, in older seedcores, protein loss was not accompanied by an increase in free amino-N. This suggests that amino acids were translocated at the same rate as protein was hydrolyzed; however, insoluble-N of older seedcores increased 67 % faster than protein was lost. Immobilization of protein during sprouting is implicated by the rapidly decreasing protein/insoluble-N ratio (Fig. 5b).

Davies (1984) concluded that intersprout competition does not lower the amino acid content (mg/g dry wt) of the developing sprouts. Yet, shoots from 19-

month-old seedcores contained 25 % (mg/shoots) and 22 % (mg/g dry wt) less free amino-N after 24 days of growth than those from 7-month-old seedcores (Table 1), and this N fraction accounted for the difference in total-N content and concentration of the shoots from the two ages of seedcores. Seedcore age may affect shoot free amino-N through its effects on shoot metabolism. Compared with excised shoots from 7-month-old seedcores, those from 19-month-old cores exhibited a lower ability to reduce and incorporate N taken up from a nutrient solution to the free amino form (Knowles, 1986).

At planting, 19-month-old seedcores contained 31 % more free amino-N than 7-month-old seedcores (Fig. 4). In older seedcores, free amino-N and soluble protein-N accounted for 30 and 26 % of total-N, respectively, a 6 % higher and lower proportion of total-N compared with that of younger seedcores. It thus appears that a gradual breakdown of protein to free amino acids occurs in tubers over a 19 month storage interval. This is supported by the lower soluble protein/ free amino-N ratio in older cores compared with younger cores at zero time (Fig. 5a). In a related study, seedcores from unsprouted 31-month-old tubers were found to contain 2.7 mg/g dry weight soluble protein-N and 4.94 mg/g dry weight free amino-N. These values are 32 % lower and 18 % higher than the protein and free amino-N content respectively, of the 19-month-old seedcores used in this study. Also, the calculated soluble protein/ free amino-N ratio for the unsprouted 31-month-old seedcores was 0.55, 43 % lower than that for unsprouted 19-month-old seedcores. It is apparent that advancing tuber age is correlated with loss of soluble protein and subsequent amino acid increase.

In summary, vigor (accumulated dry matter) of individual shoots from from 19-month-old seedcores is limited by competition among the multiple shoots for available N reserves. Also, sink strength of the individual shoots from older seedcores is greatly diminished in comparison with that of the single shoot from younger seedcores. Age-related differences in the ability of plants from older cores to obtain N during growth may be due to less foot development, and/or direct effects of meristem age on plant N metabolism. Translocation of seedcore-N reserves to developing sprouts occured at the same rate in both 7 and 19-month-old tubers. Interconversions between the various N fractions during sprout development depended on seedcore age, and in older seedcores, soluble protein-N (mg/g dry wt) was lost faster than in younger seedcores, apparently due to conversion to the insoluble fraction.

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CHAPTER IV

DIFFERENCES IN RESPIRATORY METABOLISM DURING SPROUTING OF AGED POTATO SEED-TUBERS

Introduction

Prior to sprout growth, the respiration rate of mature potato tubers averages 3 to 10 µl O_2 /h/g fresh weight (17). However, with the onset of sprouting, and the associated mobilization and transfer of nitrogen and carbohydrate reserves to the developing sprouts, respiration increases. Respiratory increases ranging from 50% (3) to 400 to 500% (11) have been reported for tubers and sprouts combined. In desprouted tubers, Isherwood and Burton (11) found the increase to be only 30% above the basal rate. Higher respiration during sprouting is necessary to meet the increased energy demands associated with this metabolically active phase of development.

Whole-tuber respiration utilizes carbohydrate as the respirable substrate (12) and can be stimulated by cyanide (30). In contrast, respiration of freshly cut tuber slices is inhibited by cyanide (13,29), uses lipids as respirable substrate (21) and is 3 to 5-fold greater than respiration of the intact tuber. Upon slicing, cyanide resistance thus disappears and respiration increases in response to wounding. Rapid degradation of cellular membranes in sliced tissue results in an increase in free fatty acids which inhibit glycolysis (26), as well as mitochondrial function (4), and which serve as the respirable substrate (19). Membrane integrity thus appears essential for cyanide-resistant respiration, and breakdown of membrane lipids with slicing results in loss of the alt pathway (31).

When tuber slices are incubated in aerated aqueous medium for 24 h, respiration reverts back to the conventional respiratory pathway (12) and cyanide resistance is regained (8). Repair of cellular membranes during the incubation period decreases the free fatty acid content, resulting in restoration of glycolysis and the Krebs cycle, and carbohydrates again become the respirable substrate (19). The 4- to 5-fold increase in respiration during the incubation period (18) is not due to activity of the alt pathway but rather to increased electron flux through the cyt chain (32). It has been suggested that biosynthesis and insertion of new lipoprotein components into the mitochondrial membrane occurs during the incubation period and results in a functional alt pathway (19).

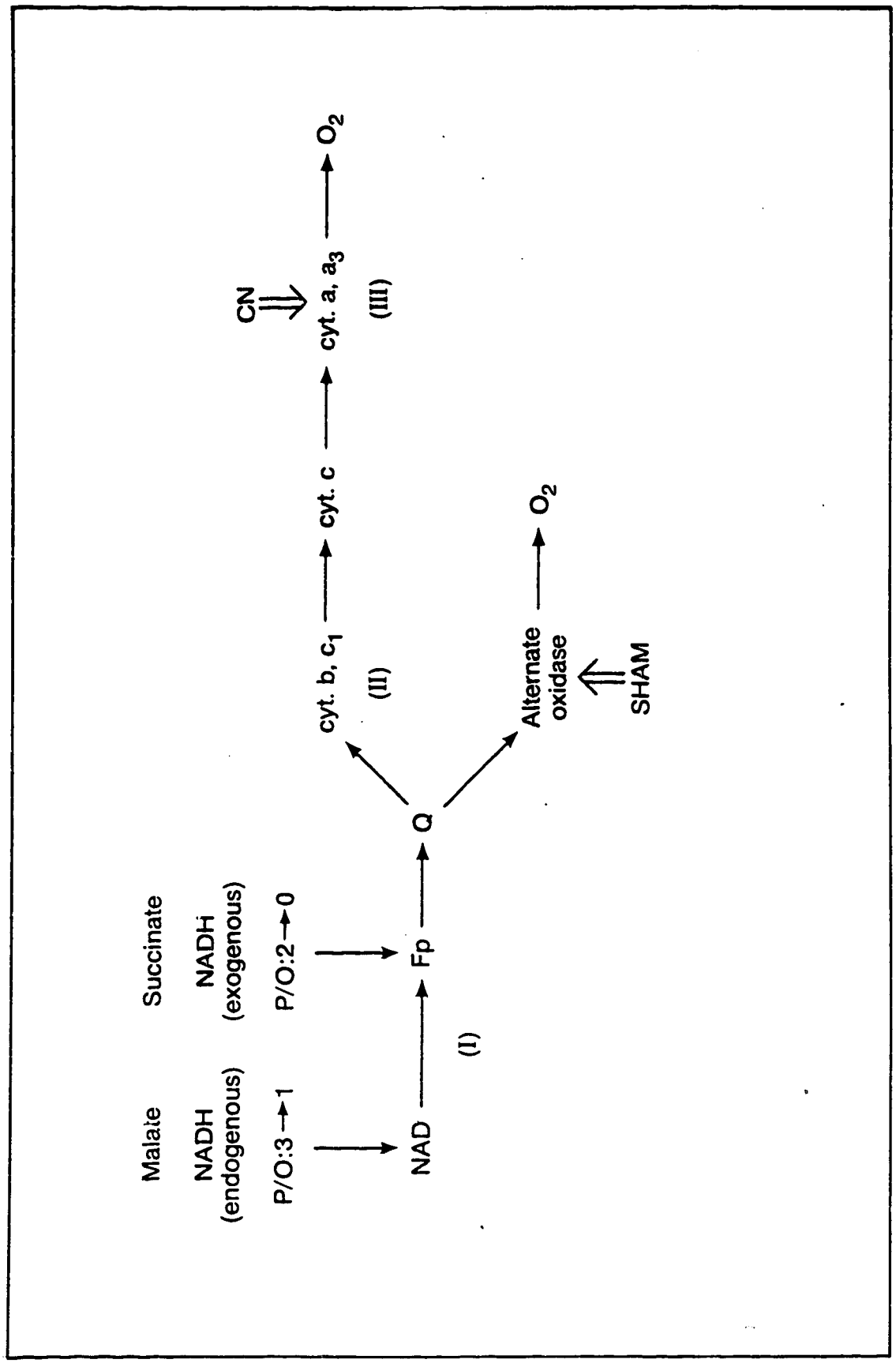
Furthermore, existence of the alt pathway in whole untreated tubers appears to be a prerequisite for alt pathway induction with aging of tissue slices, or with ethylene treatment (30).

The alt pathway is believed to be present in intact potato tubers, but is limited in use until ethylene is provided (29, 30). Treatment with ethylene greatly enhances whole-tuber respiration (30), as well as respiration of tissue slices from treated whole tubers (29). The rise in respiration with ethylene treatment is specifically due to increased flux of electrons through the alt pathway (32). Ethylene does not inhibit cyt oxidase (2) and therefore the mechanism by which ethylene stimulates the Alt pathway is not by simply attenuating electron flow through cyt oxidase. Rather, ethylene may be activating respiratory dehydrogenases (2) or inducing changes in the physical characteristics of the mitochondrial membrane which activate the alt pathway by enhancing electron transport therein. Alternatively, ethylene may be facilitating the bridge between the cyt-mediated and alt pathways (30). It is relevant to note that ethylene is produced by potato tissue for approximately 12 h after slicing (24). Endogenous wound-induced ethylene and exogenously applied ethylene may therefore act in a similar fashion in inducing the reappearance of the alt pathway. However, this has been questioned by Janes and Weist (13).

Tissue slices provide a convenient system for monitoring the different modes of respiration over long-term storage. Van der Plas et al. (36) measured respiration of slices from tubers stored at 7°C over a period of 12 months from harvest. After 5 months of storage, respiration had stabilized at a rate which was approximately half that at the time of harvest. Cytochrome oxidase activity declined slowly after 5 months, and cyanide-resistant respiration doubled over the 12-month storage period. In a similar study, cyanide resistant respiration increased linearly through 7 months of storage (35).

An increase in the proportion of cyanide-resistant respiration with time in storage may relate to sprouting capacity of seed-tubers. Since the cyanide-resistant pathway bypasses the phosphorylation sites of the cyt chain (Fig. 1), its influence on the production of energy needed for reserve mobilization and transfer to sprouts may, in part, account for reduced sprout vigor during plant establishment from aged seed-tubers. The mobilization of both carbohydrate (25) and nitrogen (15) reserves appears to be less efficient during sprouting of aged

Figure 1. Simplified diagram of the respiratory pathways present in plants (from Lance, 1982).


Malate
Succinate
NADH (endogenous)
NADH (exogenous)
P/O:3→1
P/O:2→0
NAD
Fp
Q
(I)
cyt. b, c_1
cyt. c
cyt. a, a_3
O_2
CN
(II)
(III)
Alternate oxidase
O_2
SHAM

seed-tubers. Accordingly, this study was undertaken to characterize the effect of tuber age on the contribution of the cyt-mediated and alt respiratory pathways to total respiration of seed-tuber tissue during sprouting and early plant establishment.

Materials and Methods

Potato (*Solanum tuberosum* cv. Russet Burbank) seed-tubers (PVX tested, Elite III) were stored at 4°C (95% RH) for 7 and 19 months. The effect of seed-tuber age on respiratory metabolism during the sprouting and plant-establishment stages of development was characterized in separate studies involving single-eye seedcores and whole seed-tubers.

Seedcore Study

Single-eye seedcores (1.8 cm diameter, 2 cm long), cut from the middle portion of 7 and 19-month-old seed-tubers that had been taken from a 4°C storage and acclimated to room temperature for 24 h in the dark, were planted into flats containing peat. The flats were watered and placed in a 23°C dark storage. After 6 d of growth, the seedcores were harvested, rinsed in distilled water, and a 0.9 cm diameter x 2 cm long core was cut from the center of each seedcore directly below the sprout. The smaller cores were rinsed in distilled water and sliced into 1 mm thick disks. The disks were incubated for 24 or 48 h in loosely capped flasks containing 5 mM $CaCl_2$, 1 mM DTT, 0.25 mM PMSF, 0.5 % (w/v) PVP and 0.15 mM chloramphenicol (all chemicals were from Sigma, St. Louis, MO) in phosphate buffer (5 mM, pH 6.5). The incubation medium was changed three times during the first 24 h and twice during the next 24 h. Freshly cut tissue disks (incubated 0 h) were held in incubation medium and respiratory measurements were completed within 2 h of cutting. Tissue disks from tubers that had been acclimated to 23°C for 24 h in the dark served as unsprouted (0 DAP) controls.

Respiration rate was quantified with a YSI Biological Oxygen Monitor by placing tissue disks (5 disks per treatment) into a sample chamber containing 5 ml phosphate buffer (0.01 mM, pH 7.2) and measuring the rate of oxygen consumption at 25°C. The rates of Alt and Cyt-mediated respiration were determined by first adding SHAM (10 mM final concentration) and then sodium cyanide (0.2 mM final concentration) to the sample chamber. Preliminary experiments had established that these concentrations maximally inhibited the

respective modes of respiration in potato tissue disks. Oxygen consumption occurring after the addition of both of these inhibitors is reported as res respiration. Respiratory activities are reported as nmol O_2 consumed/min/g fresh weight .

A randomized complete block design having 3 blocks and 12 treatments (2 seedcore ages x 2 harvest dates x 3 incubation times) was used to evaluate treatment effects in this study. Sprout growth and respiration were subjected to analysis of variance and, where appropriate, sums of squares were partitioned into individual degree-of-freedom components of both main effects and interactions. Based on the results of the analyses of variance, regression analysis was used to derive polynomial models describing the various relationships.

Whole Tuber Study

To compare the respiration rate of whole tubers during sprouting, 7 and 19-month-old seed-tubers taken from a 4°C storage were blocked for size, weighed and placed into 4-L glass jars (9 tubers per jar) at 23°C. The jars were sealed with metal lids having inlet and outlet ports, and were wrapped in foil to exclude light during the sprouting period. A flow board was used to deliver a uniform air-flow (23.4 L/h) to each of the jars. The inlet air was continuously passed through a diffusion stone submersed in distilled water to maintain high RH in the jars. Respiration rate of the tubers was quantified by sampling 1 ml of the outlet gas from each jar at approximately 9 h intervals during the first 3 d, and then once daily over the remaining 18 d of the study. Carbon dioxide concentration in each sample was determined with a Hewlett-Packard, model 5890A gas chromatograph equipped with a thermal conductivity detector. The 2.4 m stainless steel column (3.2 mm o.d.) was packed with 80/100 mesh HayeSep T (Hewlett-Packard). The He flow rate was 30 ml/min, and the column was isothermal at 100°C. Respiration rate of tubers was expressed as ml CO_2 /h/kg fresh weight. At the end of the study, sprout number and fresh weight were recorded.

To quantify the contributions of the different modes of respiration to total respiration during sprouting, 3 tubers of each age from 3 of the 6 replicates were removed from the jars at 6, 12 and 18 d after the commencement of the experiment. In addition, respiration of unsprouted tubers was measured using disks from tubers taken from a 4°C storage, and acclimated to room temperature for 4 h. Sprout number, length and tuber fresh weight were recorded at each

harvest and 3 single-eye cores (0.9 cm diameter x 2 cm long) were cut from the middle portion of each tuber. The cores were rinsed in distilled water, sliced into 1 mm disks and incubated for 24 h prior to quantifying the different modes of respiration as previously described (see Seedcore Study).

Results

Seedcore study

The effects of tuber age, sprouting and tissue incubation period on respiratory activity of tissue from 7 and 19-month-old seed-tubers are shown in Table 1. Total respiration of tissue incubated for 24 h was approximately 3 times that of freshly cut tissue, confirming earlier reports (13, 32, 36). Incubation for an additional 24 h (48 h total) resulted in a marginal increase in total respiration which averaged 20 % in tissue from 7-month-old cores and 42 % in tissue from 19-month-old cores. On average, sprouting for 6 d had little effect on respiration of tissue from younger seedcores. In contrast, total respiration of incubated tissue from older seedcores increased by 52 % over the 6 d interval. The respiration rate of tissue from older seedcores was also significantly greater than that from younger seedcores at 6 d after planting, reflecting a highly significant ($P<0.01$) age x harvest date interaction. In general, the increase in total respiration of tissue from 19-month-old seedcores, as a function of incubation time and sprouting, was greater than that of tissue from 7-month-old seedcores and reflected a significant ($P<0.01$) age x harvest date x incubation time interaction.

At planting, the rate of cyt-mediated respiration was equal in freshly cut tissue from the two ages of seedcores and it increased at similar rates over the 48 h incubation interval (Table 1). Cyt-mediated respiration of freshly cut tissue at 6 d after planting was also equal for the two seedcore ages, averaging 20 nmoles O_2 /h/g fresh weight. However, with increasing time of incubation, the rate of cyt-mediated respiration in tissue from sprouting (6 DAP) 19-month-old seedcores increased faster than that from 7-month-old seedcores. These trends in cyt-mediated respiration resulted in significant ($P<0.05$) age x harvest date and age x incubation time interactions.

Of particular interest was the effect of tuber age and harvest date on the rate of alt respiration. Relative to sprouted seedcores (6 DAP), alt respiration rate of tissue from both ages of unsprouted seedcores (0 DAP) was low (Table 1).

Table 1: Effect of sprouting and incubation time on respiration rate of tissue disks from single-eye seedcores from potato seed-tubers stored for 7 and 19 months.

Seedcores were planted in peat and sprouted in the dark for 6 d at 23°C. Seedcore tissue disks (0.9 cm x 1mm) were cut and incubated for 0, 24 or 48 h in phosphate buffer (5 mM, pH 6.5) containing 5 mM $CaCl_2$, 1 mM DTT, 0.25 mM PMSF, 0.5 % (w/v) PVP and 0.15 mM chloramphenical. Respiration rates were determined in phosphate buffer (10 mM, pH 7.2) at 25°C (5 tissue disks per treatment). The final concentrations of SHAM and NaCN were 10 mM and 0.2 mM, respectively. Tissue disks from tubers that had been acclimated to 23°C for 24 h in the dark served as unsprouted (0 DAP) controls.

Tuber Age (months)	Incubation Time (h)	Respiration rate (nmoles O_2 /min - g fwt)							
		Total		Cytochrome-mediated		Alternative		Residual	
		0 DAP	6 DAP	0 DAP	6 DAP	0 DAP	6 DAP	0 DAP	6 DAP
7	0	22.55	25.66	18.80	19.75	0.97	0.16	2.76	5.76
	24	69.90	71.48	56.20	52.76	1.97	5.74	11.73	12.98
	48	81.22	88.20	72.46	75.94	0.00	3.83	8.75	8.43
19	0	28.21	29.17	22.55	20.46	1.55	1.73	4.11	6.98
	24	64.12	100.07	43.31	58.30	7.49	21.52	13.32	20.25
	48	93.87	138.75	75.19	93.14	5.51	31.86	13.17	13.75
Age (A) [a]		0.01 [b]		ns		0.01		0.01	
Incubation Time $(IT)_L$		0.01		0.01		0.01		0.01	
IT_D		0.01		ns		0.01		0.01	
Days After Planting (DAP)		0.01		0.05		0.01		0.01	
A X IT_L		0.01		ns		0.01		0.01	
A X IT_D		ns		0.05		ns		ns	
A X DAP		0.01		0.05		0.01		0.05	
DAP X IT_L		0.01		0.05		0.01		0.05	
DAP X IT_D		ns		ns		ns		0.05	
A X DAP X IT_L		0.01		ns		0.01		ns	
A X DAP X IT_D		ns		ns		ns		0.05	

[a] Sources of variation (L and D subscripts indicate linear and deviations, respectively). [b] Significance levels for indicated sources of variation (ns = not significant).

The highest rate of alt respiration from unsprouted seedcores was evident when tissues were incubated for 24 h. Under these conditions, alt respiration of tissue from 7-month-old seedcores accounted for approximately 3 % of total respiration compared to 12 % for that from 19-month-old seedcores. Tissue from sprouted 19-month-old seedcores, which had been incubated for 24 and 48 h, had 3.7 and 8.3-fold higher rates of alt respiration respectively, compared with that from 7-month-old seedcores. Furthermore, as a percentage of total respiration, alt respiration of incubated tissues from sprouted older seedcores averaged 22 % compared with 6 % for that from younger seedcores.

Tissues from sprouted and unsprouted 19-month-old seedcores had slightly higher rates of res respiration compared with those from 7-month-old tissues at all incubation times. However, the proportion of total respiration accounted for by res respiration was only 3.2 % (average for unsprouted tissue) and 1.3 % (average for sprouted tissue) greater in tissue from older seedcores, compared with that from younger seedcores, over all the incubation times.

Whole tuber respiration:

Whole tuber respiration was followed for approximately 500 h (Fig. 2). The respiration rate of 19-month-old tubers was 40% higher than that of 7-month-old tubers immediately following removal of the tubers from a 4°C storage. During the initial hours of the study (up to 55 h), a transitory rise in respiration rate coincided with room temperature acclimation of the tubers. The peak in respiration, and subsequent decline to the basal rate, occurred sooner in older tubers than in younger tubers. Once stable, respiration rate of 7-month-old tubers averaged 5.7 ml CO_2 /h/kg fresh weight, a rate 21% higher than that of 19-month-old tubers which averaged 4.7 ml CO_2 /h/kg fresh weight. Respiration rates remained constant for both tuber ages during sprouting (arrows indicate presence of sprouts 1 cm in length).

After 21 d of growth, the total fresh weight of sprouts from 7-month-old tubers was nearly 3-fold greater than that from 19-month-old tubers (Fig. 3, inset). Older tubers averaged 4-fold more sprouts per eye than younger tubers; however, the average fresh weight per sprout from older tubers was 92% lower compared with that from younger tubers. Sprout length was also recorded at each harvest date. For 7-month-old tubers, sprout length increased linearly to 2.2 cm by day 18. A quadratic increase in length of sprouts from 19-month-old tubers resulted in sprouts that were 27% longer than those from younger tubers by the

Figure 2. Respiration of whole 7 and 19-month-old potato seed-tubers incubated in the dark at 23°C. Arrows indicate presence of sprouts 1 cm in length.

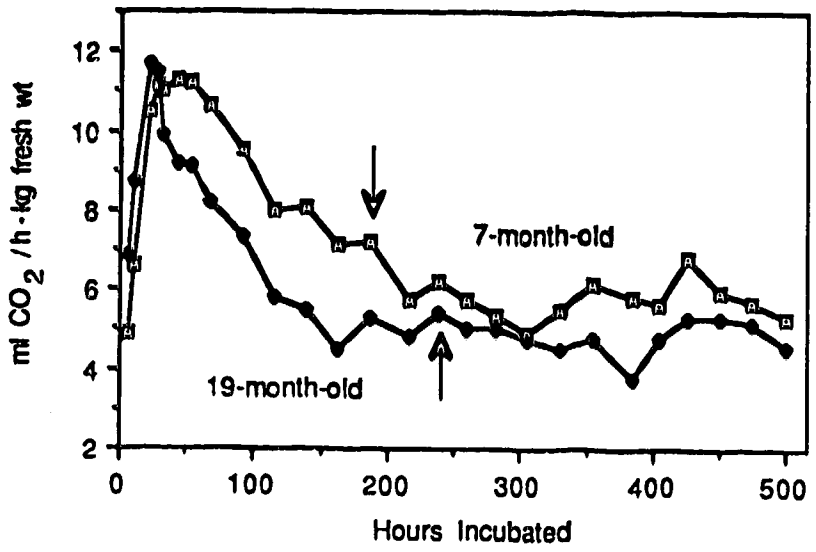
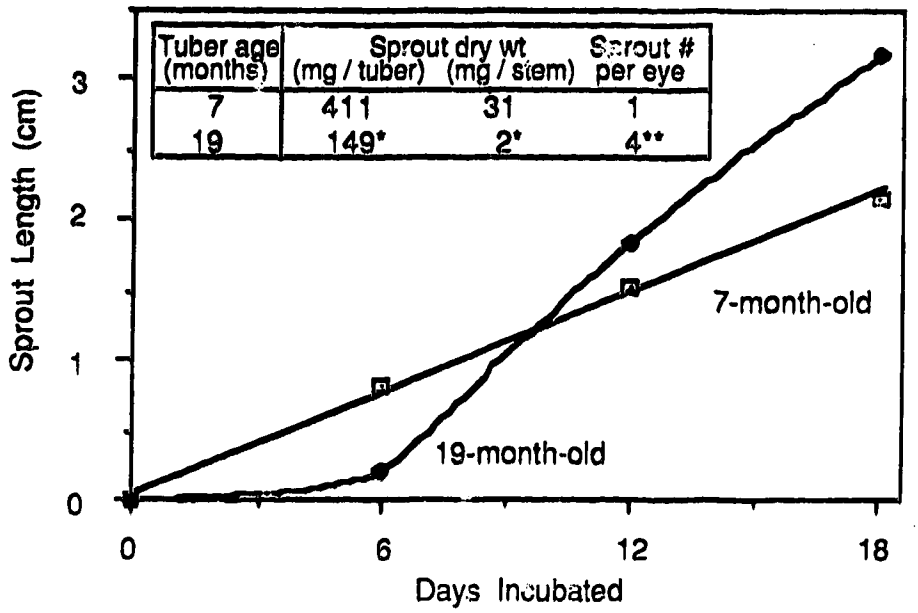
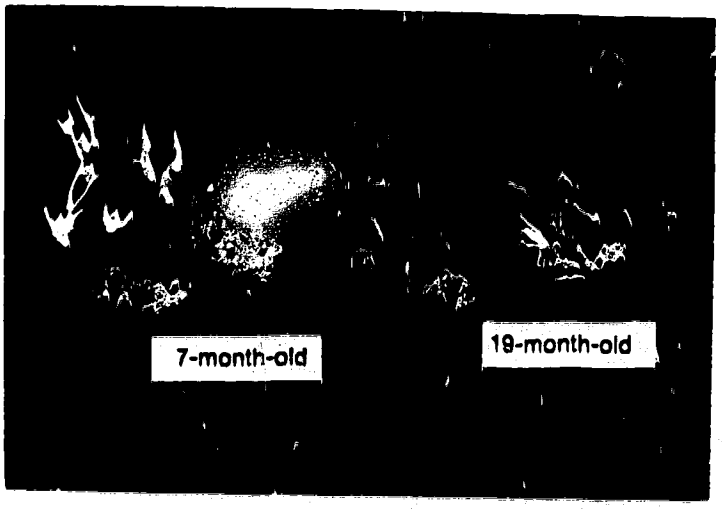
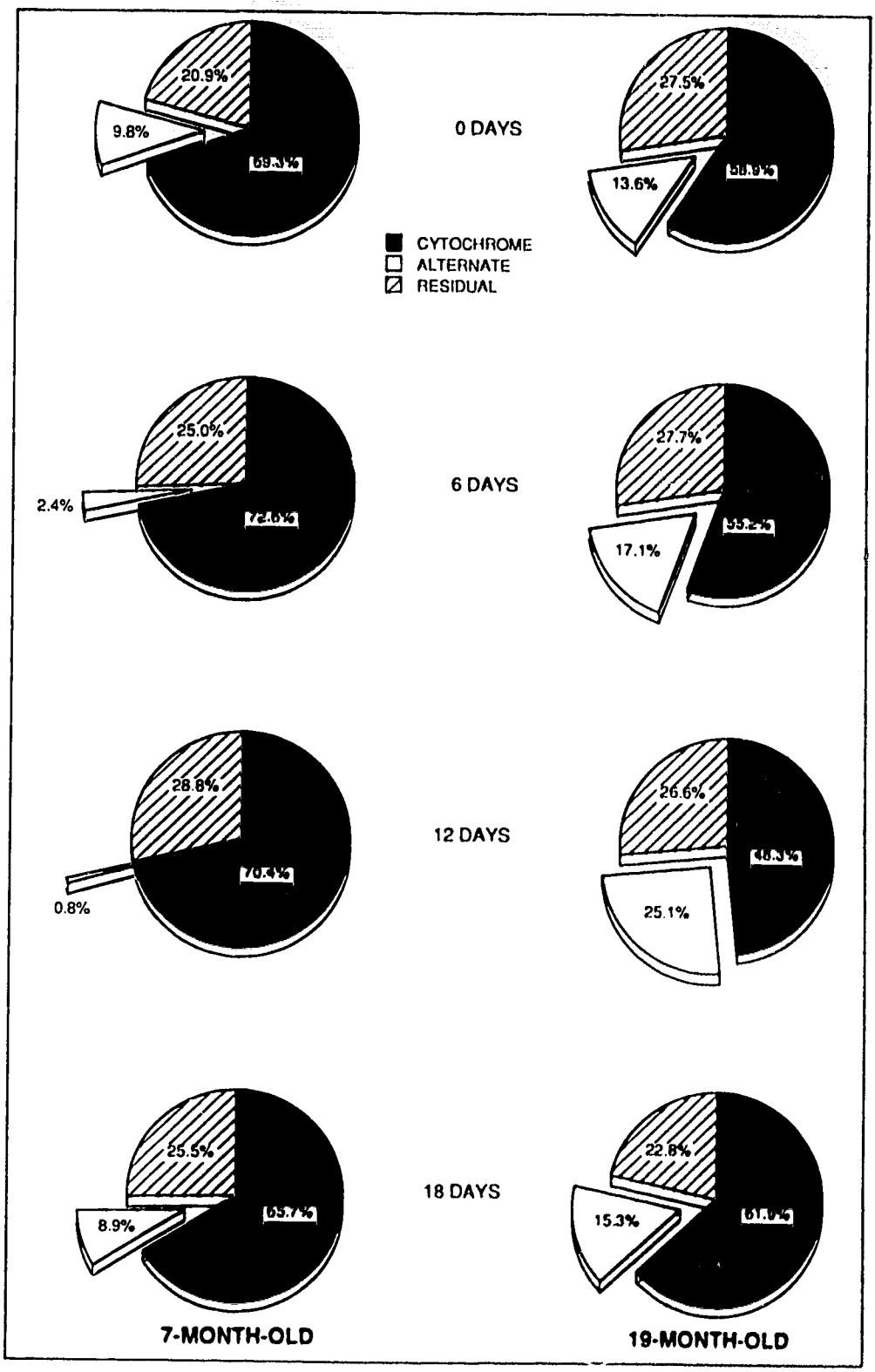

ml CO_2 /h·kg fresh wt
12
10
8
6
4
2
0
100
200
300
400
500
Hours Incubated
7-month-old
19-month-old

Figure 3. Elongation of sprouts from whole 7 (□) and 19-month-old (◆) potato seed-tubers incubated in the dark at 23°C. F-values for the main effects of age and incubation time, and the age x incubation time (linear) interaction were significant at the 0.05, 0.01 and 0.01 levels, respectively. Inset: Average dry wt and number of sprouts from 7 and 19-month-old seed-tubers after 21 d of incubation. *,**F-values for the differences between tuber ages were significant at the 0.05 and 0.01 levels, respectively.

Figure 4. Sprouted 7 (a) and 19-month-old (b) potato seed-tubers which were incubated in the dark at 23°C for 21 d. Sprouting capacity of these tubers is quantified in Figure 3.


Tuber age (months)
Sprout dry wt (mg / tuber)
(mg / stem)
Sprout # per eye
7
411
31
1
19
149*
2*
4**
Sprout Length (cm)
0
1
2
3
0
6
12
18
Days Incubated
7-month-old
19-month-old


7-month-old
19-month-old


20.9%
9.8%
69.3%
0 DAYS
27.5%
13.6%
CYTOCHROME
ALTERNATE
RESIDUAL
25.0%
2.4%
6 DAYS
27.7%
17.1%
28.8%
0.8%
12 DAYS
26.6%
25.1%
25.5%
8.9%
65.7%
18 DAYS
22.8%
15.3%
7-MONTH-OLD
19-MONTH-OLD

end of the study period. However, prior to day 10, when sprouts were of equal length, sprouts from 19-month-old tubers were significantly shorter than those from 7-month-old tubers. The elongation rate of sprouts from younger tubers thus remained constant at 1.2 mm/d, while that from older seed-tubers increased with time. These results illustrate a significant, negative affect of advanced seed-tuber age on sprouting capacity and growth potential. The age-reduced differences in sprout vigor are clearly evident in Figure 4.

Changes in respiratory activity of incubated tuber tissue from the sprouting 7 and 19-month-old seed-tubers are shown in Figure 5. The trend in total respiration was quadratic in tissue from both tuber ages, and the main effect of age was significant ($P<0.05$). Total respiration rate was equal in tissue from the two tuber ages at zero-time, but it increased faster in older tubers, reaching a level 51 % higher than that displayed by younger tubers by the end of the study. In contrast, the rate of Cyt-mediated respiration was not affected by tuber age, averaging 18 nmols O_2 /min/g fresh weight during the study (Fig. 5). Alt respiration of tissue from 19-month-old tubers increased through day 12, reaching a maximum that was 30-fold greater than that of tissue from 7-month-old tubers at the same time (Fig. 5). Conversely, alt respiratory activity in tissue from younger tubers decreased, from a zero-time rate of 2.4 nmoles O_2 /min/g fresh weight to less than 1 nmole O_2 /min/g fresh weight by day 12. The trend in res respiration was also quadratic for both ages of tissue. Throughout the study, res respiration of tissue from older tubers was 28% higher than that from younger tubers.

The contribution of the different pathways of respiration to total respiration is quantified in Figure 6. Res respiration accounted for 26 % of total respiration, and was not affected by time or tuber age. These results are in agreement with those obtained previously (32). On average, cyt-mediated respiration in tissue from older seed-tubers accounted for a significantly lower percentage of total respiration (about 56%) than that from younger seed-tubers (about 70%) throughout the study. Furthermore, in the older tissue, the proportion of cyt-mediated respiration declined by 10% (to 48% of total) from zero-time to day 12, and this occurred with a concomitant increase in alt respiration. Alt respiration increased to 25% of total by day 12 in older tissue. In contrast, alt respiration decreased from 10 to 1% of total by day 12 in tissue from younger tubers.

Figure 5. Respiration of tissue from 7 and 19-month-old potato seed-tubers sprouted in the dark at 23°C. *,**F-values for the indicated sources of variation were significant at the 0.05 and 0.01 levels, respectively.

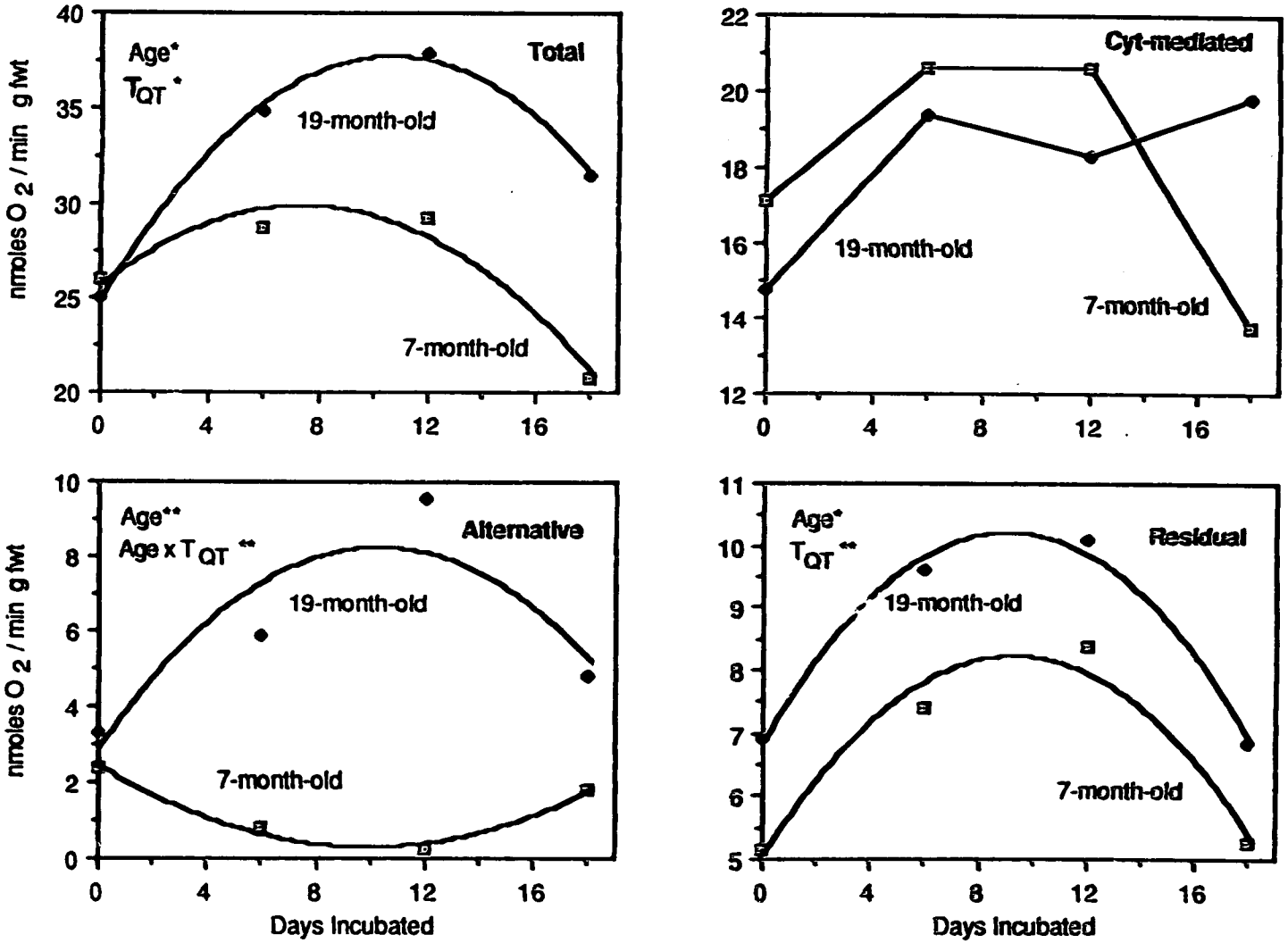

Age*
T_{QT} *
Total
19-month-old
7-month-old
nmoles O_2 / min g fwt
Cyt-mediated
19-month-old
7-month-old
Age**
Age x T_{QT} **
Alternative
19-month-old
7-month-old
Days Incubated
Age*
T_{QT} **
Residual
19-month-old
7-month-old
Days Incubated

Figure 6. Cyt-mediated, alt and res respiration of tissue from sprouting 7 and 19-month-old seed-tubers presented as a percentage of total respiration. F-values for the main effect of age, and the age x time (quadratic) interaction, were significant at the 0.01 and 0.05 levels, respectively, for both cyt-mediated and alt respiration. Changes in res respiration were not significant.

Discussion

The influence of tuber age on respiratory activity of whole tubers and tuber tissue during early sprout development was characterized in an attempt to relate potential differences in energy metabolism to reduced growth potential. The respiration rate of whole, 7- and 19-month-old seed-tubers was followed over a 500 h sprouting interval at 23°C (Fig. 2). At zero-time, older tubers were respiring at a rate approximately 40% greater than younger tubers. This initial difference in respiratory activity may be linked to a higher concentration of respirable substrate in the older tubers as a result of the senescent sweetening phenomenon (10). In previous studies, 19-month-old Russet Burbank seed-tubers were found to contain a 50% higher concentration (mg/g dry wt) of reducing sugars compared with 7-month-old seed-tubers (25).

A psuedoclimacteric in seed-tuber respiration occurred during the first 150 h of incubation (Fig. 2). This response has been well characterized as resulting from the temperature change (Q_{10}), and temperature-dependent sugar-to-starch conversions occurring within the tubers (10). Increased carbohydrate metabolism is a sink for ATP and respiration increases to maintain energy charge within the tissue. Once the tubers had acclimated to the 23°C incubation temperature, respiration stabilized at a relatively low rate during sprouting. These results were expected in light of a similar study which demonstrated that respiration was maintained at a constant low rate during the first 3 weeks (or 500 h) of sprout growth (3). Respiration does not generally increase until sprout weight is approximately equal to 1% of tuber fresh weight. By the end of the 500 h study, sprout weight from the 19-month-old tubers was only 0.6% of tuber fresh weight, while that from 7-month-old tubers was equivalent to 1.1% of tuber fresh weight.

Age-related differences in respirable substrate, degree of coupling of oxidative phosphorylation to electron transport, and/or mode of respiration may account for differences in total respiratory output during the sprouting period. Previous studies have indicated that carbohydrates are the respirable substrates in both tuber ages during sprouting (14). Moreover, experiments utilizing uncoupling agents have shown that the degree of coupling of oxidative phosphorylation is apparently similar in sprouting tubers of both ages (14). Regardless of the mechanism, the age-related differences in total respiration during sprouting are associated with differing rates of sprout development (vigor)

as characterized in Figures 3 and 4, and may be due to differences in respiratory activity of the sprouts themselves. The data in Figure 2 are a composite of both sprout and tuber respiration.

Studies aimed at characterizing respiratory activity in tissue from the two seed-tuber ages during sprouting were conducted. In agreement with previous findings (35, 36), advancing chronological age (increasing time in storage) of potato tubers was accompanied by an increase in the alt respiratory pathway (Table 1). Cyt-mediated respiration, on the other hand, was not affected by tuber age (Table 1, Fig. 5). The functional integrity of the cyt electron transport chain thus appears to persist with increasing age of potato tubers, as it does in senescing leaves (33) and ripening fruits (28).

In freshly harvested potato tubers, respiration is stimulated by ethylene, with high concentration and long exposure eliciting maximal response (27). Ethylene treatment also stimulates the alt respiratory pathway in potato tuber tissue, but only after treatment for 24 h (see reviews 5, 17, 20). Synthesis of a protein factor in the cytoplasm has been suggested as the time dependent process involved (27).

Interestingly, as tuber age advanced from 7 to 19 months, endogenous ethylene content increased by 30 % to approximately 125 ppb (25). This concentration is considered to be physiologically active in many fruits and vegetables (Abeles, 1973). Alt respiration in freshly cut tissue from 19 month old tubers was 1.6 fold higher than in that from 7 month old tubers and this difference was magnified with increasing incubation time (Table 1). Duncan and Spencer (6) have shown that the level of alt respiration in isolated mitochondria from the cotyledons of ethylene treated pea seedlings increases with increasing ethylene concentration. It is not known if the magnitude of expression of the alt pathway is ethylene concentration dependent in intact potato tubers. However, in separate experiments it was shown that ethylene content increased with sprouting and the concentrations were much greater in older than in younger seed tubers (Kumar and Knowles, unpublished). Furthermore, the level of alt pathway capacity in potato tuber callus tissue has been shown to to be controlled by endogenous ethylene (7). Notwithstanding the possibility that aging also enhances the sensitivity of the tissue to ethylene (34), it thus seems likely that increased alt pathway activity in tissue from older tubers may be invoked, at least in part, by higher levels of endogenous ethylene both before and during sprouting.

In aged soybean seeds, a marked loss of total respiratory activity, deterioration of the cyt-mediated pathway, and the engagment of the alt pathway occur during the early stages of germination. Leopold and Musgrave (22) associated these events with loss of growth vigor. Unlike aged soybean seeds, total respiratory activity of 19-month-old tuber tissue during early sprout development was greater than that of tissue from 7-month-old tubers, yet the cyt pathway was operating at a similar capacity in tissue from both tuber ages (Fig. 5). Hence, the elevated respiration rate of tissue from older tubers during sprouting was due to a higher amount of SHAM-sensitive respiration. Respiration via the alt pathway yields only one third of the energy (ATP) produced by the cyt-mediated pathway because only one phosphorylation site is engaged (Fig. 1, 20). Since the two tuber ages have the same level of cyt-mediated respiration, and older tubers have a significantly higher level of alt respiration, older tubers are generating higher levels of ATP during sprouting. However, sprouts from older tubers were less vigorous than those growing from younger tubers (Figs. 3 and 4). The rate of cyt-mediated respiration was sufficient to support the vigorous sprout growth from younger tubers, and therefore the data suggest older tubers are using available energy inefficiently during sprouting, or are using energy for metabolic processes not directly involved with sprout growth.

In potato tubers, the main translocatable sugar is sucrose, and its biosynthesis (an effective ATP sink) is directly related to the level of respiration (10, 30). Solomos and Laties (30) noted that the higher the respiration rate, the greater the accumulation of glucose and sucrose. Because the total respiration rate was greater in tissue from sprouting older tubers in this study (Fig. 5), a similar relationship in sugar content would be expected between the two tuber ages. Indeed, in a related study, reducing sugars accumulated during the early stages of sprouting of 19-month-old seed-tubers at a 2-fold higher rate than in 7-month-old seed-tubers, while sucrose content decreased (25). Starch content decreased at the same rate in 7 and 19-month-old tubers with sprouting. These results suggest that older tubers are either less efficient at synthesizing sucrose or have higher rates of sucrose hydrolysis than younger tubers during sprouting. Reduced vigor of sprouts (Figs. 3 and 4) and elevated respiratory activity (indicating rapid utilization of generated ATP) in older tubers, together with the observed age-related alterations in tuber carbohydrate metabolism during early

sprout growth (25), suggest sucrose is indeed formed, but its translocation is limited.

Solomos and Laties (30) reported that with ethylene induction of the alt pathway, the glycolytic pathway is enhanced and sucrose content of the tissue increased with starch-to-sucrose interconversion acting as the sink for ATP. This relationship may hold true during the sprouting of aged potato tubers; however, increased enzymatic hydrolysis of the newly formed sucrose would result in less translocatable carbohydrate and may account, in part, for the reduced vigor of sprouts from aged tubers. Age-related differences in tuber carbohydrate metabolism may thus relate to differences in respiratory metabolism.

Compared with 7-month old seed tubers, a greater proportion of the metabolic energy generated during the sprouting of 19 month old seed tubers may also be directed toward maintenance of membrane integrity. Maintenance of membrane integrity is an active process, and it is axiomatic that efficient reserve mobilization and energy metabolism during sprouting are dependent upon a high degree of membrane integrity in the seed-tuber tissue. Membrane deterioration is a characteristic of aging and senescence in plant tissues in general. In potato tubers, cellular membrane integrity declined linearly from 6 to 24 months of storage and this was correlated with an increase in the degree of saturation of membrane fatty acids (16). During sprouting of aged tubers, the role of increased alt pathway activity may thus be a compensatory one, to meet the increased energy demands of maintaining membrane integrity.

In conclusion, the appearance of the alt respiratory pathway in plant tissues is usually related to the phenomenon of aging or a change in physiological state (9). The alt pathway is operative in potato tubers stored for 7 months (Table 1, Fig. 5). Prolonged storage (19 months) induces additional changes in the tissue (possibly ethylene related) which contribute to the strong expression of the alt pathway, along with reduced sprout vigor and plant growth potential. This study demonstrated that the rate of cyt mediated respiration did not decrease with advanced tuber age or sprouting. Moreover, older tubers had a significantly higher rate of alt respiration compared with younger tubers and the contribution of the alt pathway to total respiration was as high as 25 %. Hence during sprouting, older tubers appear to be generating higher levels of ATP through a combination of the cyt mediated and alt pathways compared with younger tubers; however, the increased energy metabolizing ability of older tubers is less

efficient at meeting the demands of sprout growth as evidenced by the lower weight of sprouts produced. Older tubers apparently require more energy for metabolic processes not directly involved in sprout production (eg. maintenance of membrane integrity, altered carbohydrate metabolism). The nature of the difference in metabolic sinks between sprouting young and old seed-tubers remains to be established.

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CHAPTER V

POLYAMINE METABOLISM OF POTATO SEED-TUBERS DURING LONG-TERM STORAGE AND EARLY SPROUT DEVELOPMENT[1]

Introduction

In plants, the amino acids arginine and ornithine provide the main carbon skeletons for synthesis of the PAs Put, Spd and Spm. The biosynthetic pathway for these PAs is summarized in Figure 1. Interestingly, SAM is a precursor which is shared by both PAs and ethylene. Conversion of SAM to ACC leads to ethylene production, but once SAM is decarboxylated by SAMDC, it is committed to PA synthesis (Galston and Kaur-Sawhney, 1987). The syntheses of ethylene and PAs are thus interdependently related (Smith, 1985). Though ethylene and PAs share a common intermediate, they have opposite physiological effects: ethylene promotes senescence, while PAs delay senescence (Altman and Bachrach 1981).

Polyamines have been reported to have important growth-regulating properties in plants. Kaur-Sawhney et al. (1982) showed that cells in a rapidly-growing phase of development are very rich in PAs. Polyamine titer increased dramatically with the breaking of dormancy in Jerusalem artichoke (*Helianthus tuberosus* L.) tubers (Bagni et al.,1980) and during sprouting of potato tubers (Kaur-Sawhney et al., 1982). Furthermore, increased plant vigor in several species has been directly correlated with high PA titer (Smith and Davies, 1985).

The biological activity of PAs is most likely attributed to the cationic nature of the molecules, which facilitates their interaction with cellular anions such as nucleic acids and membrane phospholipids. The mechanism through which PAs influence growth and development is unknown, but they may exert a direct influence on nucleic acid and protein synthesis or on membrane stabilization. For a review of PA metabolism in plants, see Galston, 1983; Slocum et al., 1984 and Smith, 1985.

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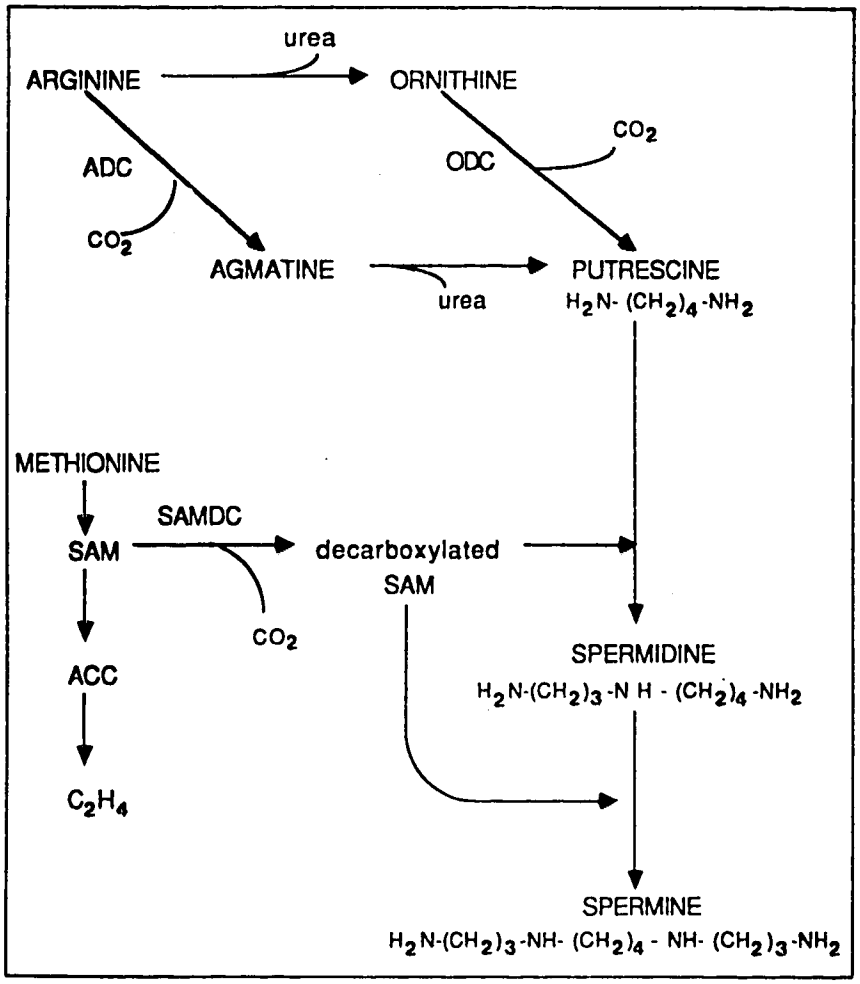
[1]Published in *Plant Physiol* (1989) 91: 183-189.

Figure 1. Interrelationship between the ethylene and PA biosynthetic pathway in plants as modified from Galston (1983).


urea
ARGININE
ORNITHINE
CO_2
ADC
ODC
CO_2
AGMATINE
PUTRESCINE
urea
H_2N- $(CH_2)_4$ -NH_2
METHIONINE
SAMDC
SAM
decarboxylated
SAM
CO_2
SPERMIDINE
ACC
H_2N-$(CH_2)_3$ -N H - $(CH_2)_4$ -NH_2
C_2H_4
SPERMINE
H_2N-$(CH_2)_3$-NH- $(CH_2)_4$ - NH- $(CH_2)_3$-NH_2

Sprouting of potato seed-tubers involves an increased rate of nucleic acid and protein synthesis which leads to plant growth and development. Age of the seed-tuber can greatly influence subsequent growth: advanced tuber age (e.g. greater than 12 months from harvest) often yields less vigorous plants. Since PAs are known to both stimulate growth and deter senescence, this study was initiated to determine the effect of long-term storage on PA content of potato seed-tubers and also to characterize the effect of tuber age on PA metabolism during sprouting and early plant establishment. Changes in PA content are related to reduced vigor of plants from aged seed-tubers and data in light of previously published results (Knowles et al., 1985; Knowles, 1986, 1987; Mikitzel and Knowles, 1989).

Materials and Methods

Plant Material, Storage and Culture

Potato (*Solanum tuberosum* L., cv. Russet Burbank) seed-tubers (PVX-tested, Elite III) were stored at 4°C (95% RH). Changes in PA titer, as a function of storage duration, were characterized by analyzing cores (1.8 cm diameter) cut longitudinally (apical to basal) from 7, 19 and 31-month-old tubers. Cores from 5 tubers of each age were collectively sliced, frozen at -20°C and lyophilized. The lyophilized tissue was ground in a mortar and pestle and the free PAs were analyzed as described below. There were 4 replicates of each tuber age (20 tubers analyzed per age).

For plant growth and development studies, 7 and 19-month-old seed-tubers were removed from storage, acclimated to room temperature for 24 h in the dark and blocked for size. Single-eye-containing cores (1.8 cm diameter) were then cut from the middle portion of the tubers perpendicular to the long (apical to basal) axis as previously described (Knowles, 1987). A 2 cm long core was cut from both ends of the main cores such that one contained a single eye (seedcore) and the other only periderm (residual core). Apical and basal cores were avoided. Core fresh weights were recorded, seedcores were planted 3 per pot and the corresponding 3 residual cores were sliced thinly, frozen at -20°C and lyophilized for zero-time (at planting) PA determinations.

Seedcores were planted at a depth of 5 cm into 15 cm diameter pots containing a sterile, nutritionally inert medium of peat:vermiculite (1:2, v/v). The pots were placed in a growth chamber with 25/18 °C day/night temperatures in a randomized complete block design with 4 blocks and 8 treatments (2 seed-

tuber ages x 4 harvest dates). A combination of cool-white fluorescent and incandescent bulbs provided 450 $\mu E\ m^{-2}\ s^{-1}$ light intensity for 16 h per day. Pots were watered as needed and plants were harvested 12, 16, 20 and 24 days after planting. At each harvest, portions of the fresh shoot and seedcore tissues were used to assay activities of the PA biosynthetic enzymes directly. The remaining tissues were frozen at -20°C, lyophilized and used to assay content of the free PAs.

Extraction and Assay of Free PAs

Lyophilized tuber and shoot tissues were ground in a Wiley mill (40 mesh screen). Extraction of free PAs was accomplished by grinding (mortar and pestle) 225 mg of either tissue in 1 ml of 10% (v/v) PCA at 4 °C. The crude homogenates were incubated for 1 h on ice, then centrifuged for 15 min at 26,000g (4°C). The free PAs within the resulting supernatants were separated by TLC as described below.

Standards, consisting of 1 mM each of Put, Spd and Spm (Sigma), along with the PCA-soluble extracts, were dansylated according to Flores and Galston (1982), and 20 µl of each extract was loaded on the pre-adsorbent zone of high-resolution silica gel TLC plates (Whatman LK6D). The chromatogram was developed for approximately 1 h in chloroform:triethylamine (25:2, v/v). Following TLC, location of the derivatized PA bands was established in UV light; the PAs were then scraped from the plates, eluted in 2 ml ethyl acetate, and quantified using a Perkin-Elmer fluorescence spectrophotometer (model 650-10LC). Emission at 500 nm was monitored after excitation at 350 nm.

Enzyme Assays

Fresh tuber and shoot tissues were extracted (mortar and pestle) at 4°C in 100 mM phosphate buffer (pH 7.6). The extraction ratio was 5 g tissue/1.5ml buffer. The homogenate was centrifuged (26,000g, 15 min, 4°C) and activities of ADC, ODC and SAMDC within 100 µl of supernatant were analyzed according to Kaur-Sawhney et al. (1982). ODC activity was determined by measuring the $^{14}CO_2$ released from the substrate DL-(1-^{14}C)ornithine (49 mCi/mmol; NEN). Similarly, ADC and SAMDC activities were measured using L-(U-^{14}C)arginine (324 mCi/mmol; NEN) and (carboxyl^{14}C)SAM (60 mCi/mmol; NEN). The reaction mixtures contained 10 µl of 20 µCi/ml of each nuclide, diluted with unlabeled L-

ornithine, L-arginine or SAM to yield final concentrations of 66, 9 and 2.7 mM, respectively.

Enzyme assays were carried out in 3.7 ml glass vials fitted with serum stoppers. A filter-paper disk (7 mm diameter), impregnated with 50 µl of 2 N KOH, was suspended inside each vial from the serum stopper to trap the $^{14}CO_2$ liberated. For all three enzyme assays, the reaction mixture (containing 100 µl crude enzyme and 10 µl of the appropriate substrate) was incubated for 45 min at 37°C. The reaction was stopped by injecting 200 µl of 10% (w/v) TCA into each vial. The vials were incubated for an additional 45 min to facilitate quantitative trapping of the $^{14}CO_2$. The filter-paper disks were removed, immersed in 2 ml of ScintiVerse E (Fisher) and the radioactivity liberated was determined by counting in a Packard Minaxi B Tri-Carb 4000 liquid scintillation counter. Enzyme activity was expressed as nmol $^{14}CO_2$ liberated/h/g fresh weight.

Internal Ethylene Extraction and Assay

The internal ethylene concentration of tubers stored 7, 19 and 31 months (4°C, 95% RH) was quantified by GC utilizing a photoionization detector (Bassi and Spencer, 1985). Whole tubers were blocked for size and sliced; internal gases were extracted from the slices utilizing a vacuum extraction method (Beyer and Morgan, 1970). The method was modified so that the tissue was submersed in a saturated solution of Na_2SO_4 in the extraction apparatus. This reduced the solubility of ethylene, thus increasing the accuracy of detection (Beyer and Morgan, 1970). The submerged tissue was subjected to a constant vacuum of 200 mm Hg for 2 min. Ethylene content of the extracted gas sample was analyzed with a Photovac model 10A10 portable GC fitted with a photoionization detector (Photovac Inc., Thornhill, Ontario, Canada). A 244 x 3 mm teflon column, containing Carbopack B (60-80 mesh)/1.5% x E-60/1.0% H_3PO_4 (Supelco Canada Ltd), was used with purified air (15 ml/min) as the carrier gas. The GC was run at ambient temperature. Two experiments, each involving three replicates of each age, were performed.

Statistical Procedures

A randomized complete block design was used in each of the studies reported herein. Plant growth, PA titer and PA biosynthetic enzyme activities were subjected to analysis of variance and, where appropriate, sums of squares were partitioned into individual degree-of-freedom components of both main

effects and interactions. Based on the results of the analyses of variance, regression analysis was used to derive polynomial models describing the various relationships.

Results

Changes in Tuber PA and Ethylene Content During Storage

The effect of long-term storage on PA content of potato seed-tubers is presented in Figure 2a. As tuber age advanced from 7 to 31 months, significant linear trends in Put, Spd and Spm content were observed. Put increased 2.2-fold while Spd and Spm decreased 33 and 38%, respectively. In addition, tissue ethylene concentration was found to increase by 60% as tubers aged from 7 to 31 months (Fig. 2b).

Growth and Development from Aged Seed-tubers

Single-eye-containing cores from 7 and 19-month-old seed-tubers were sown and plant growth was compared over a 24 day interval. Changes in PA titer and biosynthetic enzyme activities in both seedcore and plant tissues were also followed. Shoot and root dry-matter accumulation (mg/core) are shown in Figure 3. The trend in total shoot dry matter (Fig.3a) from both core ages was quadratic; however, the growth rate of plants from younger cores was significantly greater than that from older cores, reflecting a negative effect of advanced seed-tuber age on plant vigor. After 24 days of growth, 7-month-old cores had produced 64% more shoot dry matter than 19-month-old cores. Similarly, root growth of plants developing from younger cores was 50% greater than that from older cores after 24 days (Fig. 3b). Root growth of plants from younger cores was linear, while that from older cores was quadratic. In addition to the negative effect of advanced seed-tuber age on plant vigor, an influence on the partitioning of plant dry matter is illustrated by the root-to-shoot ratios (Fig. 3b, inset), where a highly significant ($P<0.01$) age x time interaction was characterized. In 7-month-old seedcores, roots were produced at a faster rate than shoots during the initial stages of establishment, resulting in a root/shoot ratio greater than 1. In contrast, the rate of shoot growth from older seedcores exceeded the rate of root growth throughout the study, as indicated by a root/shoot ratio consistently less than unity. Furthermore, the root/shoot ratio of plants from younger seedcores decreased while that from older seedcores increased over the growth period.

Figure 2. Effect of chronological age on free-PA (a) and internal ethylene content (b) of potato seed-tubers stored at 4°C and 95% RH. F-values for the linear effect of age on Put, Spm and ethylene content were significant at the 0.05 level and for Spd at the 0.01 level.

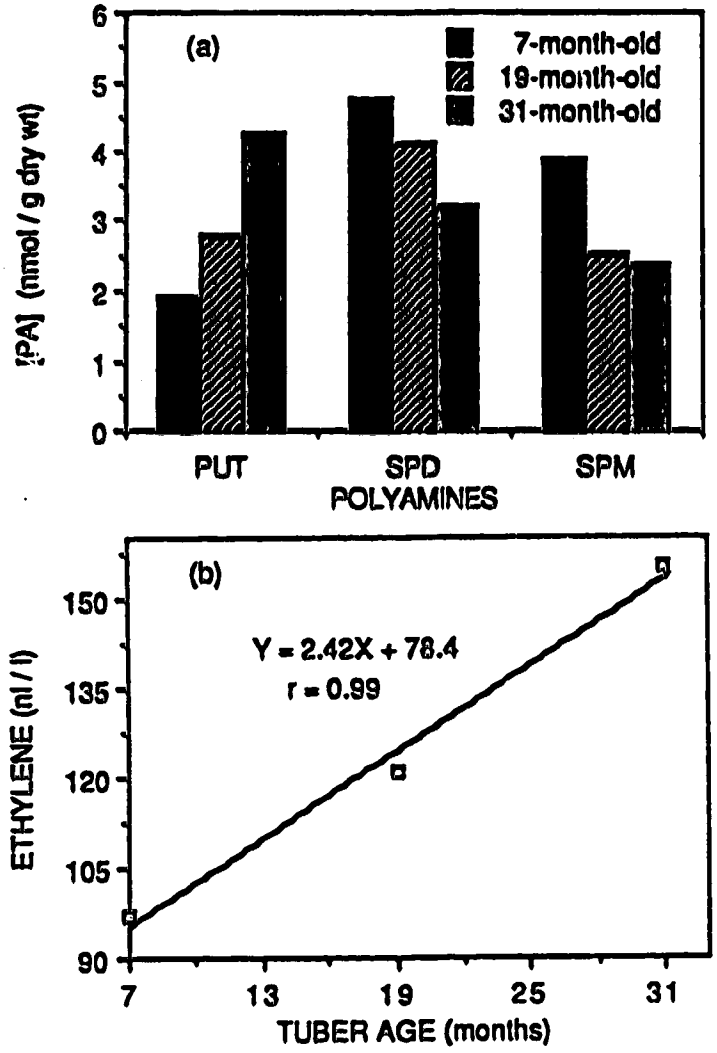
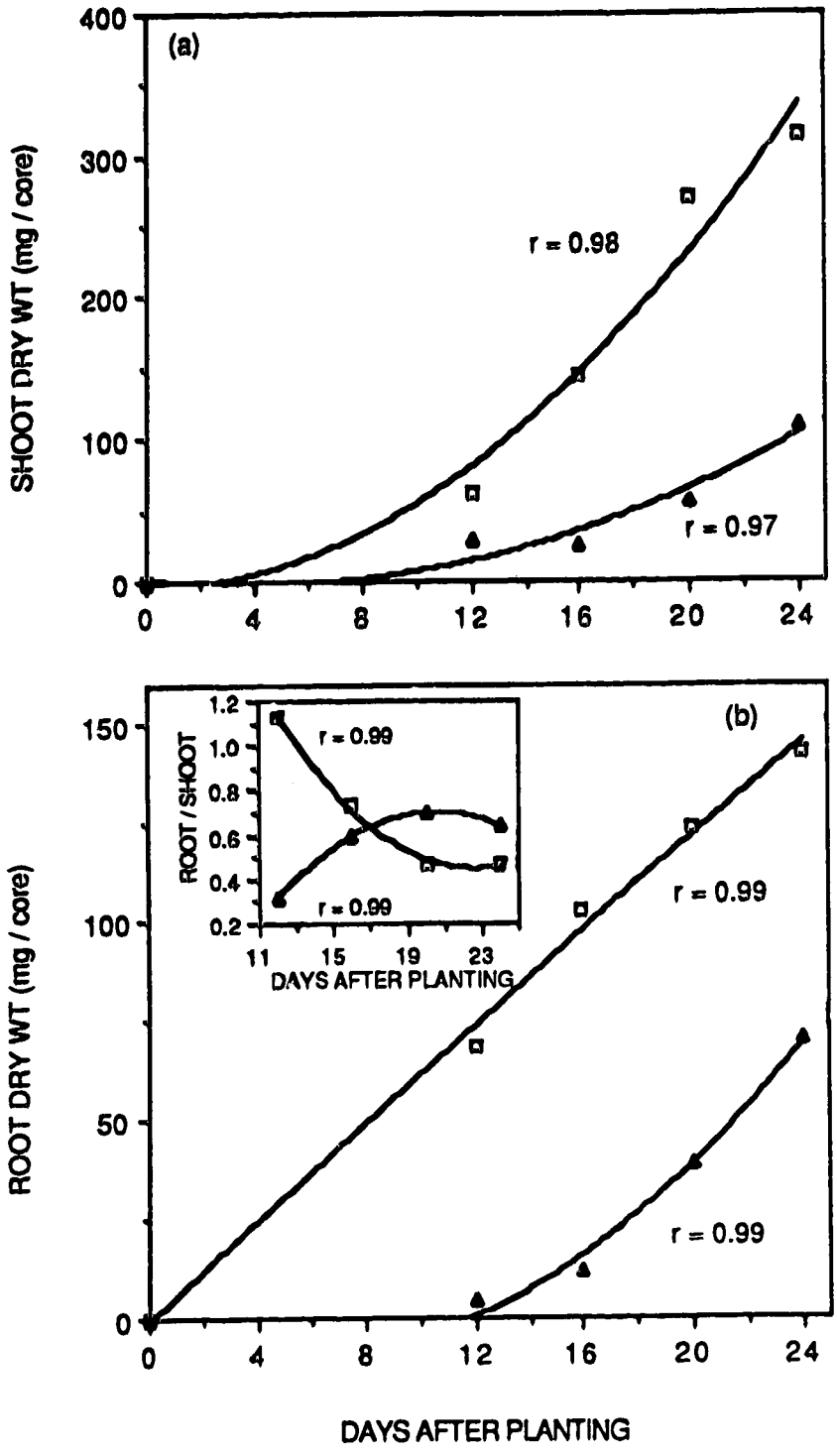

(a)
7-month-old
19-month-old
31-month-old
[PA] (nmol / g dry wt)
0
1
2
3
4
5
6
PUT
SPD
SPM
POLYAMINES
(b)
Y = 2.42X + 78.4
r = 0.99
ETHYLENE (nl / l)
90
105
120
135
150
7
13
19
25
31
TUBER AGE (months)

Figure 3. Time course of shoot dry weight (a), root dry weight (b) and root to shoot dry weight ratio (inset) from single-eye seedcores from 7 (□) and 19-month-old (▲) seed-tubers. (a) F-value for the interaction of seedcore age x time (linear) was significant at the 0.01 level. (b) and inset, F-values for the interaction of seedcore age x time (linear and quadratic) were significant at the 0.01 level.


(a)
SHOOT DRY WT (mg / core)
400
300
200
100
0
r = 0.98
r = 0.97
0
4
8
12
16
20
24
(b)
ROOT DRY WT (mg / core)
150
100
50
0
r = 0.99
r = 0.99
ROOT / SHOOT
1.2
1.0
0.8
0.6
0.4
0.2
r = 0.99
r = 0.99
11
15
19
23
DAYS AFTER PLANTING
DAYS AFTER PLANTING

PA Metabolism During Development

Change in Put titer (nmol/g dry weight) of seedcore tissue over the 24 day growth period is shown in Figure 4a. In 7 month-old cores, Put content increased 7-fold, reaching a maximum of 14 nmol/g dry weight by day 14; this was 100% higher than the maximum (7 nmol/g dry weight) reached in older seedcores (an increase of 2.8-fold over the initial Put content). Like Put, seedcore Spd content (Fig. 4 b) changed quadratically over the 24 day growth period. In younger cores Spd content almost doubled during the first 12 days of growth and then decreased through day 24. In older cores, Spd titer decreased slightly over the first 8 days of growth, then slowly increased to a level approximately 21% higher than the initial content at planting. Spermidine content of 19-month-old cores was significantly lower than that from 7month-old cores throughout the study. The main effect of time and the age x time interaction were not significant for seedcore Spm content. The main effect of age, however, was highly significant ($P<0.01$). Younger seedcores contained 37% more Spm than older cores (3.20 nmol/g dry weight compared with 2.33 nmoles/g dry weight) throughout the study.

The activities of the PA biosynthetic enzymes were measured in seedcore tissue at 12 and 16 days after planting to coincide with the maximum content of Put and Spd observed in both core ages (Table I). Seedcore age and harvest time had no effect on ADC activity, which averaged 0.14 nmol/h/g fresh weight. From 12 to 16 days after planting, ODC activity decreased 45% in older core tissue. In contrast, the decline in ODC activity from younger core tissue during the same interval was only 6%, illustrating an age x time interaction ($P<0.10$). Furthermore, SAMDC activity in older seedcore tissue increased 321% from day 12 to 16, while in younger seedcores a 15% decline in activity was evident over the same 4 day interval.

The PA content of shoots developing from 7 and 19 month old seedcores was measured at 16, 20 and 24 days after planting. The concentrations of Put (Fig. 5a) and Spd (Fig. 5b) were significantly higher in shoots growing from older cores than from younger cores. However, seedcore age had no effect on Spm titer (Fig. 5c). All three PAs decreased linearly with time, and the rate of decrease was not affected by the age of the seed tubers from which the seedcores were taken. Paradoxically, the activities of the PA biosynthetic enzymes were lower on average, in shoots from older cores than in those from younger cores (Fig. 6)

Figure 4. Change in concentration of Put (a) and Spd (b) in single-eye seedcores from 7 (□) and 19-month-old (▲) seed-tubers during plant establishment. F-values for the interaction of age x time (quadratic) were significant at the 0.01 and 0.10 levels in (a) and (b), respectively. The main effect of seed-tuber age on both PAs was significant at the 0.01 level.

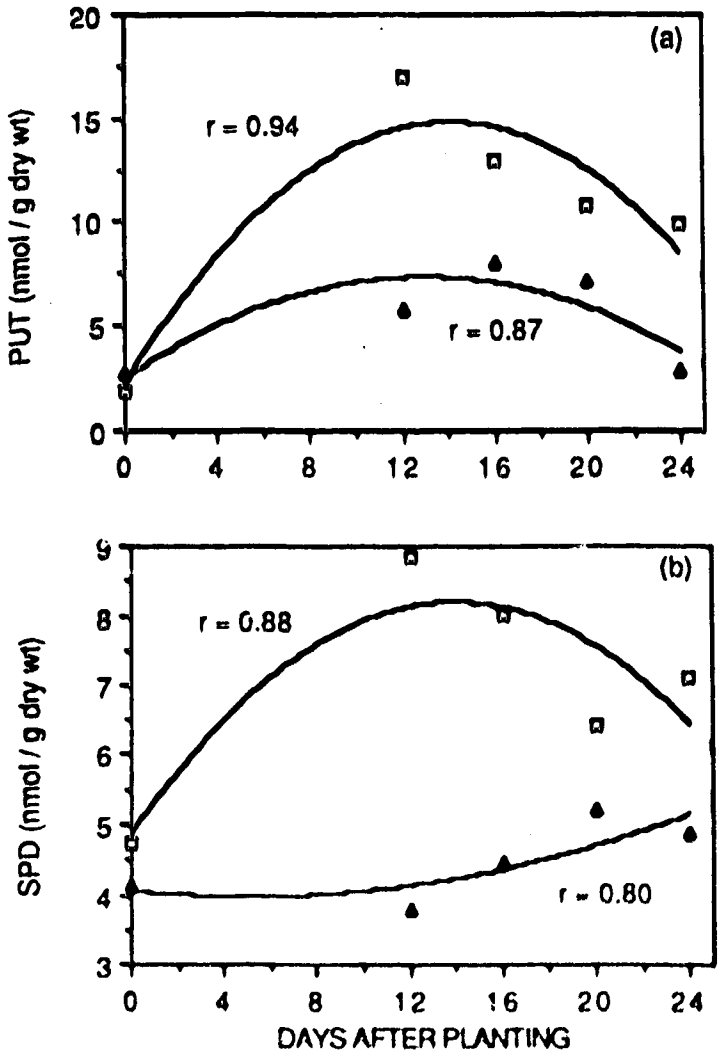

(a)
r = 0.94
r = 0.87
PUT (nmol / g dry wt)
20
15
10
5
0
0
4
8
12
16
20
24
(b)
r = 0.88
r = 0.80
SPD (nmol / g dry wt)
9
8
7
6
5
4
3
DAYS AFTER PLANTING

Table 1: Effect of seed-tuber age on the activities of ADC, ODC and SAMDC in tuber tissue at 12 and 16 days after planting.

The reaction mixtures contained 0.1 ml enzyme preparation in 100 mM phosphate buffer (ph 7.6) with the appropriate substrate (see Materials and Methods). The reaction mixtures were incubated for 45 min at 37°C and the reactions were stopped by injecting 200 ul of 10% (w/v) TCA into each of the mixtures. The data representing each treatment is the average of 12 seedcores (4 blocks x 3 seedcores per treatment).

Tuber Age (months)	Days After Planting	Decarboxylase Activities (nmol CO_2 / h /g fresh weight)		
		ADC	ODC	SAMDC
7	12	0.11	3.43	6.72
	16	0.13	3.21	5.70
19	12	0.17	4.78	3.45
	16	0.17	2.63	11.09
Age[a]		ns	ns	ns
Time		ns	0.05	0.01
Age x Time		ns	0.10	0.01

a Indicated sources of variation were either significant at the levels shown or were not significant.

Figure 5. Change in concentration of Put (a), Spd (b) and Spm (c) in shoots developing from single-eye seedcores from 7 and 19-month-old seed-tubers. *,**F-values for the indicated sources of variation were significant at the 0.05 and 0.01 levels, respectively.

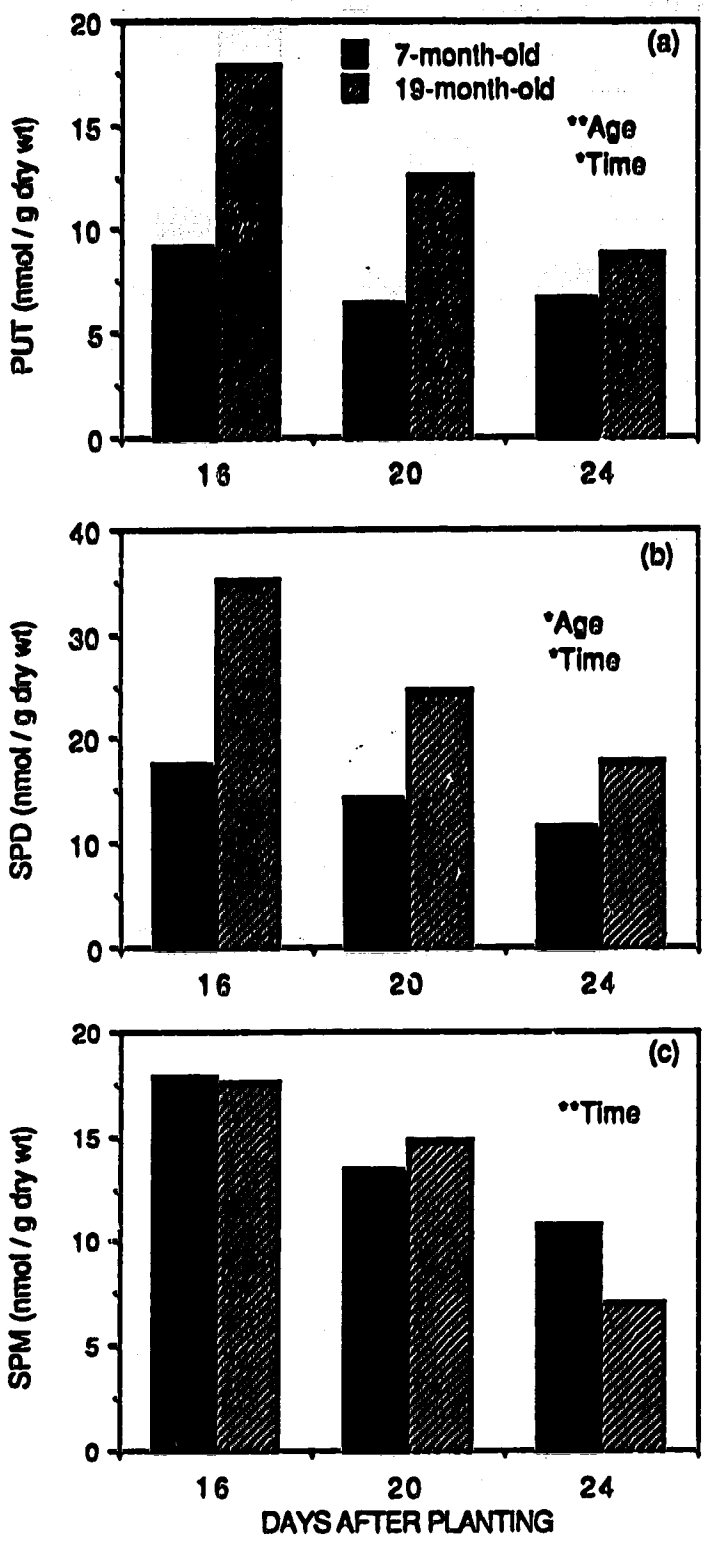
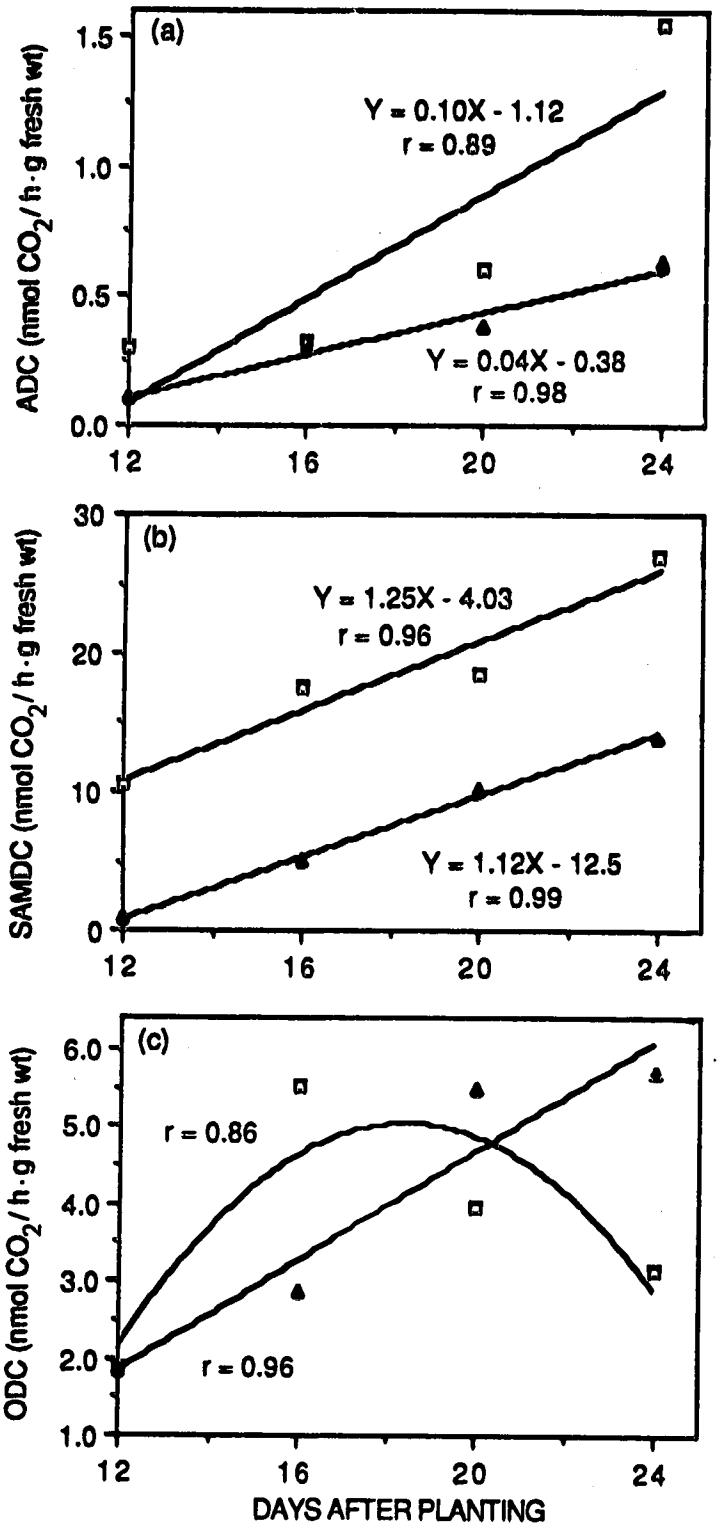

7-month-old
19-month-old
(a)
**Age
*Time
PUT (nmol / g dry wt)
20
15
10
5
0
16
20
24
(b)
*Age
*Time
SPD (nmol / g dry wt)
40
30
20
10
0
16
20
24
(c)
**Time
SPM (nmol / g dry wt)
20
15
10
5
0
16
20
24
DAYS AFTER PLANTING

Figure 6. Changes in the activities of ADC (a), SAMDC (b) and ODC (c) of shoots developing from single-eye seedcores from 7 (□) and 19-month-old (▲) seed-tubers. Details of the enzyme assay are the same as in Table 1. F-values for the following sources of variation were significant - (a) age (0.01 level), time linear (0.01) and age x time linear (0.05); (b) age (0.01), time linear (0.01); (c) time linear and quadratic (0.01 and 0.05, respectively) and age x time linear (0.05).


(a)
Y = 0.10X - 1.12
r = 0.89
Y = 0.04X - 0.38
r = 0.98
ADC (nmol CO2 / h·g fresh wt)
1.5
1.0
0.5
0.0
12
16
20
24
(b)
Y = 1.25X - 4.03
r = 0.96
Y = 1.12X - 12.5
r = 0.99
SAMDC (nmol CO2 / h·g fresh wt)
30
20
10
0
12
16
20
24
(c)
r = 0.86
r = 0.96
ODC (nmol CO2 / h·g fresh wt)
6.0
5.0
4.0
3.0
2.0
1.0
12
16
20
24
DAYS AFTER PLANTING

Arginine decarboxylase activity increased linearly in shoots developing from both seedcore ages; however, the rate of increase was 250% greater in shoots from younger cores than in those from older cores (compare slopes in Fig. 6a). Similarly, SAMDC activity increased linearly in shoots growing from both seedcore ages; however, the rate of increase was equal (compare slopes in Fig. 6b), and shoots from the younger cores had twice the activity of those from the older cores. A significant age x time interaction ($P<0.01$) characterized ODC activity (Fig. 6c). In shoots growing from older cores, ODC activity increased linearly with time; a quadratic trend characterized shoots growing from younger seedcores.

Discussion

As in cut carnation flowers (Roberts et al. 1984) and rice seeds (Mukhopadhyay et al., 1983), aging of potato seed-tubers is accompanied by the accumulation of Put (Fig. 2a). The fact that during long-term storage Spd and Spm titer decreased (while their direct precursor Put increased) suggests less efficient conversion of Put to Spd and Spm with advancing tuber age (see Fig. 1). Reduced activity or de novo synthesis of Spd and Spm synthase or limited availability of decarboxylated SAM may be responsible. The increase in ethylene concentration within tubers aged from 7 to 31 months (Fig. 2b) supports the possibility that as potato tubers age, SAM is directed toward ethylene synthesis at the expense of the PA pathway. This would result in a reduction of Spd and Spm levels with a concomitant increase in internal tuber ethylene. Since SAM is a precursor to both ethylene and the tri- and tetra-amines, it appears to be the pivotal point in determining which pathway is completed. Application of exogenous Put or Spd inhibits ethylene production at the SAM to ACC step (Even-Chen et al., 1982). Furthermore, PA synthesis can be blocked by treatment with ethylene, which inhibits SAMDC (Apelbaum et al., 184). In vivo, however, the control mechanism which switches SAM from PA to ethylene production is still unknown. The results lend further support to the idea proposed by Roberts et al. (1982) that the onset of senescence of plant tissue may be controlled in part by competition between the ethylene and PA biosynthetic pathways for SAM.

Single-eye seedcores were cut from 7 and 19-month-old seed-tubers and planted. The objective was to relate changes in PA content of seedcores and developing plants to age-reduced vigor as characterized in this and previous studies (Knowles, 1986,1987; Knowles et al.,1985; Mikitzel and Knowles, 1989).

Younger seedcores produced an average of 1.5 shoots per eye, compared with 4.3 shoots per eye from older seedcores. In addition, after 24 d of growth, older cores produced a significantly lower amount of total shoot dry matter (Fig. 3a). Hence, on a per-shoot basis, the dry matter produced from older cores was 88 % lower than that from younger cores, illustrating the effect of seed-tuber age on plant vigor.

In both ages of seedcores, Put and Spd titer increased with sprouting, albeit to a substantially lesser extent in older cores (Fig. 4). During sprouting of seedcores from older tubers, the low levels of Spd and Spm (see Results) may simply reflect the lower Put content of the cores soon after planting, since Put is a direct precursor of both Spd and Spm.

The reduced ability of older cores to increase their PA titer during plant establishment (Fig. 4) is coincident with reduced growth potential, as characterized in Fig. 3. Bagni et al. (1980) suggested that the increase in PAs with breaking of dormancy in *Helianthus* tubers induced sprouting by stimulating nucleic-acid and protein synthesis. On the other hand, Kaur-Sawhney et al. (1982) attributed second messenger roles to PAs in initiating sprouting of potato tubers. Regardless of the primary mechanism involved, changes in PA titer of seedcore tissue during early plant establishment may relate to the efficiency of mobilization of seedcore carbohydrate and nitrogen reserves to developing sprouts. For example, Srivastava et al. (1985) found that PAs increased protease activity in the cotyledons of germinating radish seeds by 40 to 50%. They postulated that PAs, by enhancing protease activity, increased reserve protein mobilization and hence growth of the seedlings. Activation of pre-existing hydrolytic enzymes or stimulation of the release of proteases were suggested as possible modes of action. Our previous studies have shown that mobilization of tuber carbohydrate and nitrogen reserves is less efficient in tubers aged for 19 months (Knowles, 1987; Mikitzel and Knowles, 1989). Furthermore, the limitation imposed on plant growth by a lower efficiency of reserve mobilization has been implicated as a contributing factor to age-reduced vigor of potato seed-tubers (Knowles, 1987; Mikitzel and Knowles, 1989). In 19-month-old potato seedcores, though PA titer does increase marginally with sprouting, mobilization of reserves may be restricted by the lower overall PA content.

Since in seedcore tissue, ADC activity was significantly lower than ODC activity and remained constant during sprouting (independently of seedcore age) (Table I), ADC may not be directly involved in Put synthesis during sprouting. In

fact, ODC has been implicated as the rate-limiting enzyme for the synthesis of Put in potatoes (Kaur-Sawhney et al., 1982). At 12 days after planting, ODC activities (Table I) in 7 and 19-month-old seedcores were statistically similar ($LSD_{0.05}$= 1.54 nmol $^{14}CO_2$/h/g fresh weight). The significant decrease in ODC activity in older cores from day 12 to 16 (Table I) may be responsible for the lower Put content relative to that of younger cores (Fig. 4a).

The dramatic increase in SAMDC activity in older cores may be in response to a lower tissue concentration of Spd and Spm. However, over the 24 day growth period, Spd and Spm titer of older cores never reached the high level evident in younger cores, perhaps because Spd and Spm were rapidly catabolized in the older cores. A more plausible explanation is that the lack of available substrate, namely Put (Fig. 4a), limited the biosynthesis of the tri- and tetra-amines, and thus their concentrations remained lower in older tissue during development. Interestingly, SAMDC is activated by Put in *Vinca rosea* (Baxter and Coscia, 1973) but not in corn (Suzuki and Hirasawa, 1980) or mung bean (Coppoc et al., 1971). It is unlikely that SAMDC is a Put-activated enzyme in potato, since in older cores, Put titer was low (Fig. 4a) while SAMDC activity was high (Table I). However, the possibility that SAMDC is stimulated by low levels of Spd or Spm warrants further investigation.

In general, PA titer and activities of the PA biosynthetic enzymes are highest when cell division is most active. This has been shown, for example, in potato tubers emerging from dormancy (Kaur-Sawhney et al., 1982) and in corn root apical meristems (Dumortier et al., 1983). Also, Smith and Davies (1985) found that PA concentration is directly related to bud size and vigor in peas. Shoots from older seedcores appear to be an anomaly, they have a higher di- and triamine titer with a lower enzyme activity and greatly reduced vigor when compared with shoots from younger seedcores (Fig. 5). Furthermore, the concentration of Spm, which is considered to have a higher growth-stimulating activity than Put or Spd, was equal in shoots developing from the two seedcore ages. Because of a slower rate of sprouting of 19-month-old cores (Fig. 3), the plants produced are developmentally younger than those from 7 month-old cores. In mung bean hypocotyls, higher free polyamine titer is associated with the most actively dividing cells, and bound PAs (TCA-soluble) are associated with the less active, more differentiated cells (Goldberg and Perdrizet, 1984). Since only free PAs were measured in this study, shoots from younger cores, which are developmentally more advanced than shoots from older cores and thus have a

higher proportion of more differentiated tissues, may contain less of the free PAs and more of the bound PAs. Conversely, shoots from older cores would have more of the free and less of the bound PAs. It is therefore possible that the less vigorous shoots from older cores do not necessarily contain more total PAs than shoots from younger cores, merely more in the free form as indicated in Figure 5.

In tomato and potato plants, high ODC activity has been correlated with high mitotic division, while ADC has been implicated in cell expansion and differentiation (Cohen et al., 1982a). It follows that shoots from younger cores, which are growing at a faster rate than shoots from older cores, should have higher activities of these two enzymes on average. The quadratic trend in ODC activity in shoots from younger cores may reflect feedback regulation by its product Put (Cohen et al., 1982b).

In summary, potato seed-tuber age affects PA metabolism in both tuber and shoot tissues. Over a 31-month storage interval, seed-tuber Spd and Spm titer decreased with a concomitant build-up of Put and tissue ethylene. This would suggest that senescence of potato tubers may be partly controlled by competition between the ethylene and PA biosynthetic pathways for SAM. During plant establishment, ODC activity remained relatively constant in younger seedcores but declined 45% in older seedcores. Hence, Put content of older seedcores remained at a relatively low level during plant establishment and this most likely contributed to the reduced ability of older cores to increase Spd and Spm titer. The resulting low level of PAs in older seed-tubers was coincident with reduced growth potential.

Differences in PA titer and biosynthetic enzyme activities within shoots developing from old and young seed-tubers probably reflect differences in maturity of the developing plants. Although the evidence is largely correlative, it appears that age-reduced vigor of potato seed-tubers may be related to dysfunctions in PA metabolism within the seed-tuber and possibly within the developing plants. The results presented provide a basis for further research on the role of PAs in loss of growth potential during aging of potato seed-tubers.

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CHAPTER VI

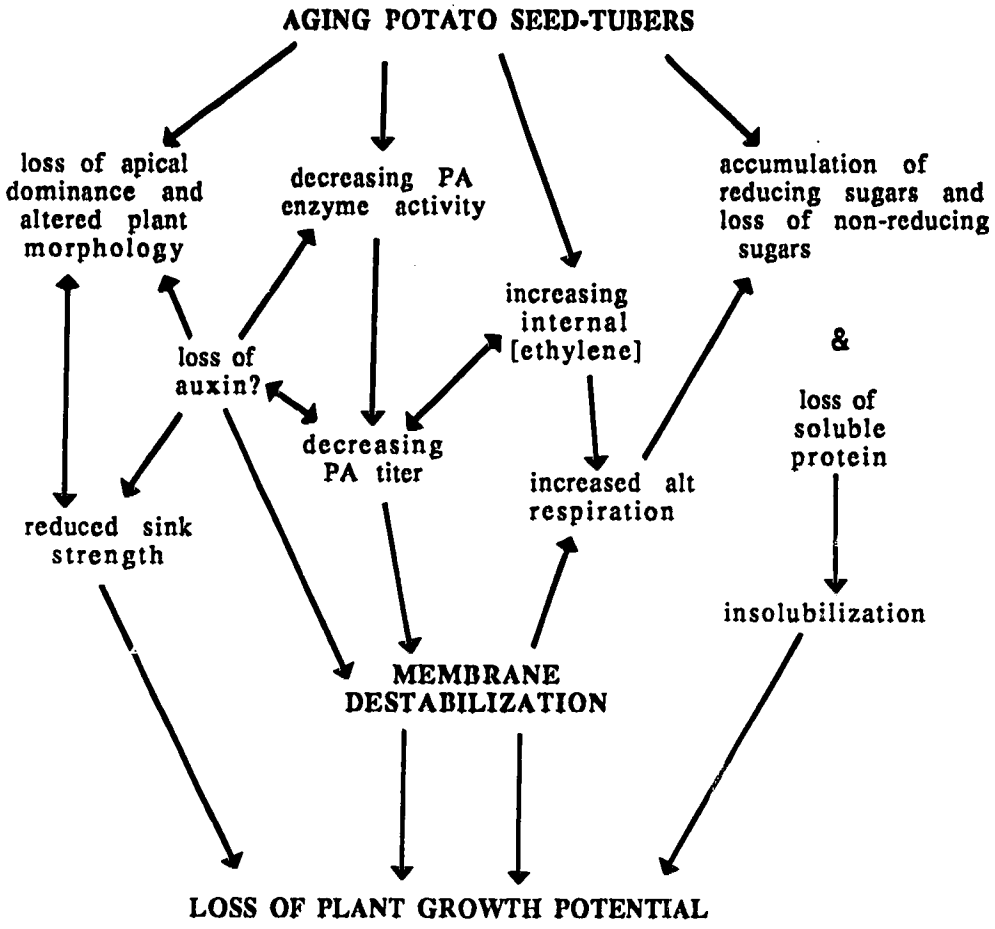
SUMMARY AND CONCLUSIONS

In relation to age-induced loss of growth potential, the influence of advancing potato seed-tuber age on respiratory activity, reserve mobilization, PA metabolism and hormone (auxin and ethylene) physiology of tuber tissue was studied. Many of the observations in aged potato tuber tissue are consistent with phenomena occuring in other senescing plant systems. For example, PA titer also decreases with age of oat leaf tissue (Kaur-Sawhney et al., 1982) and carnation flowers (Roberts, et al., 1984); there is a progressive loss of protein in aging bean leaves (Makrides and Golthwaite, 1981); mitochondrial function is maintained in senescing fruits (Romani, 1978) and alt respiratory activity increases (Solomos, 1977; Romani, 1984); and finally, decreasing auxin action and increasing sensitivity of the tissue to ethylene are generally indicative of aging.

Senescence of plant tissue is associated with membrane destabilization occurring early in the process, with catabolism of membrane phospholipids regarded as the major contributing factor. During aging of numerous plant tissues, the recorded increase in gel-phase lipid, rigidification of bulk membrane lipid and formation of nonbilayer lipid configurations are all evidence of membrane disruption (see Thompson, 1988). It has been proposed that membrane destabilization leads to the loss of selective permeability (and hence increased leakiness), proteolytic activity and generalized loss of membrane function and integrity. Thus, with increasing tissue age the regulatory influence of membranes is lost and metabolism disrupted

In this study, membrane properties were not measured directly, however, age-induced alterations of the metabolic processes studied suggest loss of membrane integrity in aged potato seed-tuber tissue (however it is mediated). Furthermore, Knowles and Knowles (1989) found that as potato seed-tubers aged in storage from 6 to 24 months membrane integrity decreased. This was correlated with an increase in the degree of saturation of membrane fatty acids, indicative of the loss of unsaturated fatty acids. The results of this study and the concept of impaired membrane function affecting metabolism due to increased tissue age are integrated in Figure 1. No attempt is made to distiguish causal

Figure 1. Schematic diagram of the possible relationship between potato seed-tuber metabolism and age-induced loss of plant growth potential.


AGING POTATO SEED-TUBERS
loss of apical dominance and altered plant morphology
decreasing PA enzyme activity
increasing internal [ethylene]
accumulation of reducing sugars and loss of non-reducing sugars
&
loss of auxin?
decreasing PA titer
increased alt respiration
loss of soluble protein
reduced sink strength
insolubilization
MEMBRANE DESTABILIZATION
LOSS OF PLANT GROWTH POTENTIAL

effects and the schematic is solely intended to illustrate the inter-relationships between the various metabolic processes studied, the loss of growth potential and the (possible) link to altered membrane function with advancing tuber age.

A model for membrane breakdown involved with plant senescence has been proposed by Leshem (1987). Increased cytosolic calcium (Ca^{2+}) combines with the calmodulin present and activates phospholipase A_2, which in turn releases the sn-2 positoned polyunsaturated fatty acids (PUFA) of the membrane. The released PUFA are subsequently catabolized by lipoxygenase to hydroperoxidizes and oxy-free radicals which then function as endogenous ionophores that enable more Ca^{2+} to enter the cell, combine with more calmodulin, and the process continues. Hence, the cascade effect of senescence is accounted for by this model. Although the nature of the initial triggering mechanism for senescence (the initial perturbation or change in the membrane which allows entry of Ca^{2+}) is not known, this sequence of reactions explains the loss of membrane fatty acids with senescence (McKersie, et al., 1978), the increased production of free radicals (Mayak, et al., 1983), and membrane rigidification attributable to free radical-mediated lipid peroxidation (Pauls and Thompson, 1980).

During the aging of potato seed-tubers, loss of apical dominance and reduced sink strength indicative of reduced auxin levels. Reduced auxin content of fruits (Coombe, 1960), leaves (Roberts and Osborne, 1981) and flower tissue (Gilbart and Sink, 1971) with increasing age has been reported, and correlated with increased IAA oxidase activity. Also, age-induced decline in auxin content may be accompanied by reduced peroxidase activity (Gilbart and Sink, 1971). The decrease in auxin, through its effect on peroxidase (an effective free radical scavenger), may thus contribute to the loss of membrane integrity with advancing age. In maize, when the influx of Ca^{2+} is restricted, rooting is stimulated over 350 % (Vaughan and Mulkey, 1985), suggesting an association between auxin and Ca^{2+} (the proposed initiator of senescence) since auxin is involved in root formation. Also, Ca^{2+} binding to membrane phospholipids which induces partial separation of the bridged molecules into discrete domains (Leshem, 1987), can be reversed by auxin application (Buckhout et al., 1981). It appears auxin is able to mediate the fluidity of membranes and decrease microviscosity, ie. stabilize membranes. Reduced sink strength and apical dominance, which leads to altered morphology (such as reduced root growth) of plants developing from aged potato seed-tubers, may be the manifestations of reduced auxin content of the tuber tissue or of auxin-membrane interactions influenced by tuber age.

Both PA and auxin are considered to be anti-senescent agents. The decline in PA titer with age may also be auxin-related since it is known that auxin stimulates the activity of PA biosynthetic enzymes (Mizrahi and Heimer, 1982). Polyamines, due to their polycationic nature, readily bind to the negatively charged headgroups of the bilayer phospholipid and selectively rigidify the membrane surface (Roberts, et al., 1986). By binding, PA are able to protect membranes from the degradative effects of phospholipases (Sechi et al., 1978), influence fluidity and indirectly modulate the activities of membrane-associated enzymes.

Any inhibition of the ethylene biosynthetic pathway leads to an increase in the production of PA (Apelbaum et al. 1981). The inhibitoy effects of PA on ethylene production have been related to the prevention of the decrease in microviscosity associated with senescence and the resultant conformational change in the ethylene-synthesizing enzyme complex (Apelbaum et al., 1981; Ben-Arie et al., 1982). However, the inhibition of ethylene producton by PA is antagonized by free Ca^{2+} (Apelbaum et al., 1981), and the ACC to ethylene step is stimulated by the presence of cytosolic Ca^{2+} (Cheverry et al., 1988). Cheverry, et al. (1988) also concluded that the ethylene forming enzyme complex is located on the inner side of the plasma membrane. The production of ethylene, which itself may alter membranes (Hansen and Kende, 1975) is favoured by the age-induced changes of the membrane structure (Leshem, 1987). Ethylene production is also enhanced by Ca^{2+}:calmodulin phospholipase activation (Leshem, 1984) and the free radicals generated through membrane-associated lipoxygenase (Legge and Thompson, 1983). In aged potato tuber tissue, ethylene content increased and PA decreased: stimulation of ethylene by cytosolic Ca^{2+} cannot be ruled out.

Mitochondrial structure and function are stable very late into the aging process, and an increase in respiration is a frequent manifestation of tissue senescence. These observations led to the hypothesis that increased respiratory activity is a compensatory or homeostatic response to the cumulative intracellular stress of senescence (Romani, 1978). The increase in alt respiration (possibly ethylene related) in aged potato tuber tissue, which did not occur in younger tissue, may be an expression of the homeostatic potential of the tissue to generate (additional) ATP for the maintenance of cellular membranes. The formation of sucrose is also an effective sink for ATP, and ethylene-stimulation of the alt pathway in potato is correlated with increased glycolytic activity and sucrose content of the tissue (Solomos, 1977). Degradation of the amyloplast membrane

that occurs with advancing age of potato tubers results in the accumulation of reducing sugars in the cytoplasm (Isherwood, 1976). Rapid sucrose formation in aged tissues may contribute to increased respiratory rates. During sprouting of aged tubers however, rapid hydrolysis of sucrose apparently limited sprout growth.

In senescing climacteric fruit (Brady et al., 1970), detached barley leaves (Atkin and Srivastava, 1970) and senescing carnation flowers (Bufler et al., 1983) the increase in protein synthesis, as measured by amino acid incorporation, is indicative of increased rates of protein turnover, and is viewed as an upsurge in metabolism. As suggested by Pech and Romani (1979) this upsurge of protein synthesis that accompanies aging may be a repair mechanism. A similar increase in protein synthesis occured in 19-month-old potato tuber tissue (Mikitzel and Knowles, unpublished). Per mg protein present, the amount of ^{14}C-leucine taken up by the tissue, and the percentage incorporated into TCA-precipitable protein during early sprout growth was approximately 265 % and 123 % greater respectively, in 19-month-old tissue compared with 7-month-old tissue. Increased uptake of the amino acid by older tuber tissue is an indication of increased membrane permeability, and an indirect measure of membrane integrity. The significantly higher rate of amino acid incorporation in older tissue suggests, as in the examples above, a repair-type mechanism. In aged soybean cotyledons, the potential for repair (recovery from senescence) exists even after 85 % of the protein has been lost (Krul, 1974). In potato tuber tissue, this potential also appears to exist, however during sprouting protein apparently becomes immobilized.

In summary, the findings of this research suggest that tuber metabolism is influenced by age-induced alterations of cellular membranes, the result of which culminates in the loss of plant growth potential (Fig. 1). The events leading to reduced plant vigor with advancing tuber age are complex and the inital process in aging and/or senescence remains to be elucidated. Nevertheless, age related metabolic dysfunctions in reserve mobilization, respiratory activity and PA and auxin metabolism contributed to the significantly reduced vigor of plants from aged potato seed-tubers.

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