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PHYSIOLOGICAL STUDIES OF POTATOES, SOLANUM TUBEROSUM L., GROWN IN ARIZONA

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# PHYSIOLOGICAL STUDIES OF POTATOES, *SOLANUM TUBEROSUM* L., GROWN IN ARIZONA

by

Ahmet Arslan

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A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN HORTICULTURE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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THE UNIVERSITY OF ARIZONA
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Ahmet Arslan

entitled Physiological Responses of Potatoes, Solanum tuberosum L. Grown in Arizona.

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Phylosophy.

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|---|---|
| Karen Mathews | July 30, 1986 |
| Paul G. Bartels | Date July 30, 1986 |
| James W. O'Leary | Date July 31, 1986 |
| Paul M. Bessey | Date July 31, 1986 |
| Norman F. Oebker | Date July 31, 1986 |

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

| | |
|---|---|
| Karen Mathews | July 30, 1986 |
| Dissertation Director | Date |

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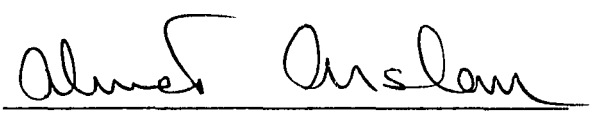
SIGNED: 

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ABSTRACT

The physiological development of potato plants and tubers grown in Arizona desert regions were examined and spectrophotometric methods were developed for the analysis of carbohydrates in potato tubers, pollen, and leaf tissues from various plants. Seed pieces were planted (March 1979, 1980) and harvested (June, July) in 2 locations. Emergence, tuber initiation, and maximum tuber numbers occurred 3, 5, and 10 weeks, respectively, after planting. In 1979, tuber yields in Mesa were low because of a shortened growing season; in Tucson, yields were drastically reduced because of soil water-logging. In 1980, tubers were planted in Mesa (March 5) and yield of the cultivar 'Kennebec' was higher, 'Denali' was nearly equal to whereas 'Norgold Russet' was lower than the national average for the respective cultivars. 'Kennebec' had the highest leaf area index (LAI) and plant height. Plantings in Marana (March 21) had lower LAI and plant height than in Mesa and yielded less, but rankings of cultivars for yield were the same. During rapid tuber growth, air temperatures often exceeded 40°C, but lower canopy temperatures were 30°C or lower, and water potentials ($\psi$) were -9 bars or higher.

Chemical analysis showed tubers increased starch percentages during development, and levels at maturity were similar to those found in other regions of the country.

Extracts for carbohydrate analysis were obtained by overnight immersion in 80% ethanol and total soluble sugars (TSS) were analyzed

by an improved anthrone assay and also with para-hydroxybenzoic acid hydrazide (p-HBAH) following methylation and permanganate oxidation. Methods were also developed for estimating total keto sugars with phenol in acetic/sulfuric acid (PASA) and also with thiobarbituric acid (TBA). Aldohexoses were determined with o-toluidine and o-ethylaniline; total reducing sugars were analyzed with p-HBAH, and sucrose was estimated with anthrone or PASA or TBA following alkaline enolization or borohydride reduction of monosaccharides. Starch was determined quantitatively after enzymatic hydrolysis to glucose and analyzing with anthrone, o-toluidine, or p-HBAH. Potential errors involved in the methods are discussed and the techniques were applied to carbohydrate analysis in plant tissues and pollen.

# CHAPTER 1

## INTRODUCTION

Chapter 2 is entitled "Physiological Responses of Potato Cultivars grwon in Desert Regions," and deals with the relationship of top to tuber grown of potato plants grown in 1979 at Tucson and Mesa, Arizona, and in 1980 at Mesa and Marana, Arizona.

Chapter 3 is entitled "Development of Spectrophotometric Methods for Analysis of Carbohydrates in Plant, Pollen, and Insect Tissues." It is specifically concerned with various analytical methods which were developed to determine various forms of soluble and insoluble carbohydrates found in plant tissues.

CHAPTER 2

# PHYSIOLOGICAL RESPONSES OF POTATO CULTIVARS GROWN IN DESERT REGIONS

## Background and Objective

Potatoes, Solanum tuberosum L., are grown mostly in the northern regions of the United States, but there are about 2,500 hectares with an annual value of $9 to $13 million currently being grown in the desert regions of central Arizona (Arizona Agricultural Statistic Bulletin, 1981). Potatoes are normally harvested in early June in Arizona, when the supplies of fresh northern potatoes are low (Pew et al., 1976). As a result, demand for high quality potatoes is high and there is potential for increasing the production of potatoes for both home consumption and the potato chip industry.

Most commercial potatoes are cool season crops that are sensitive to high temperature (Marinus and Bodlaender, 1975), water deficits (Gandar and Tanner, 1976a, 1976b; Singh, 1969), and low humidity (Harris, 1978), but they are also sensitive to temperatures lower than 16°C (Moorby and Milthorpe, 1975; Sale, 1973a, 1973b). In Arizona, yields are known to decrease considerably when plants are damaged by low winter temperatures and also when temperatures climb to high levels in mid May (Pew et al., 1976).

Potato breeders have been trying to develop cultivars that are frost tolerant during early stages of growth and heat tolerant during

tuber formation (Moorby and Milthorpe, 1975). Recently, the cultivar 'Denali' was developed in Alaska from parental stocks which had high frost resistance, high starch contents, and good yields (Dearborn, 1979). Field trials over 3 years in Nebraska and at the University of Arizona Experimental Farm in Mesa, Arizona have shown this cultivar produced tubers of excellent quality, and yields were generally good. Its growth habits differed from other cultivars and the plants had higher resistance to wilting than other cultivars.

The objective of this study was to compare the relationship of plant growth to tuber yield and quality of 'Denali' with two commercial varieties, 'Kennebec' and 'Norgold Russet', when grown in Arizona.

## Literature Review

### General Developmental Patterns of Potato Plants

Potatoes are propagated commercially, using tuber section "seed pieces" which have nondormant buds. Bud dormancy appears to occur because of high abscisic acid (ABA) levels, and during storage ABA levels drop and gibberellin levels are thought to reach levels that will permit axillary buds located in the "eyes" to become nondormant and begin elongating (Burton, 1978; Mingo-Castel, Young, and Smith, 1976). Several sprouts may originate from a seed piece, but the largest becomes dominant and inhibits the growth of others. As the emergent sprout establishes above-ground stems and leaves, it becomes photosynthetic and progressively less dependent on the seed piece for food reserves. Stolons are produced by development of buds located at

the nodes of underground portions of the stem, and tubers are initiated by the radial enlargement of the tips of these stolons (Lovell and Booth, 1969; Moorby and Milthorpe, 1975; Plaisted, 1957). Stolon nearest the seed piece normally form tubers first, and tuber size tends to decrease in the upper stolons due to dominance by the first tuber (Moorby and Milthorpe, 1975).

Tubers are initiated 2 to 3 weeks after emergence (Sale, 1973 ), but once the first flower is formed, the main stem's apical dominance is broken. In some cultivars, this leads to emergence of new stems from the underground nodes of the main stem, whereas in other cultivars stems can develop from buds located on the seed piece (Smith, 1968). Subsequently, the above-ground axillary branches on these and the main stem will continue their development and the increased leaf area that occurs is considered to provide the necessary assimilates for later tuber bulking, which is the period from the time tubers are 45 g in size until harvest (Reeve, Timm, and Weaver, 1971).

Roots of potatoes that arise from the tuber seed pieces are fibrous and branched. Roots can also arise adventitiously as groups of 3 from the internodal area of the underground stem. These roots arise near the soil surface but later turn downward after growing horizontally for some distance (Hector, 1933).

## Tuber Initiation, Anatomy, and Development

Although the apex of a young stolon is hooked, stolons and shoot apexes during primary growth are similar in that apical zones

have high numbers of meristematic cells which are subtended by elongating and vacuolated cells. Cross-sectional views show 3 large and 3 small bicollateral bundles that are limited externally by well-developed cortical tissues and internally by the pith (Artschwager, 1918; Fig. 1a). As stems and stolons differentiate, the continued development of fascicular and interfascicular cambium results in rings of outer phloem, xylem, and inner phloem (Fig. 1b). Fibers are formed in the outer phloem, whereas many cells in the inner phloem remain parenchymatus. The collection of parenchyma, phloem sieve elements, and the companion cells in the inner phloem is referred to as the perimedullary zone (Fig. 1b). Tuber development is largely due to enlargement of the parenchyma cells located in the perimedullary zone of the inner phloem (Fig. 1c), but parenchyma cells in the cortex also enlarge. In comparison to stems, stolons have relatively few xylem elements; therefore, the amount of woody tissue is limited (Cutter, 1978).

During tuberization, the first internode below the apical bud begins to thicken radially and the hook disappears when the second internode begins to thicken. Radial thickening continues downward and several older internodes will be included in the tuber (Plaisted, 1957). Tuber growth initially occurs largely because of concurrent division and enlargement of cells in the internal and external phloem and cortex. Division declines sharply when the tuber reaches 2.5 cm in diameter and a weight of about 45 g (Reeve et al., 1971), and subsequent tuber growth is associated with cell enlargement (Reeve, Timm, and Weaver, 1973). The parenchyma cells ultimately accumulate high amounts of starch and

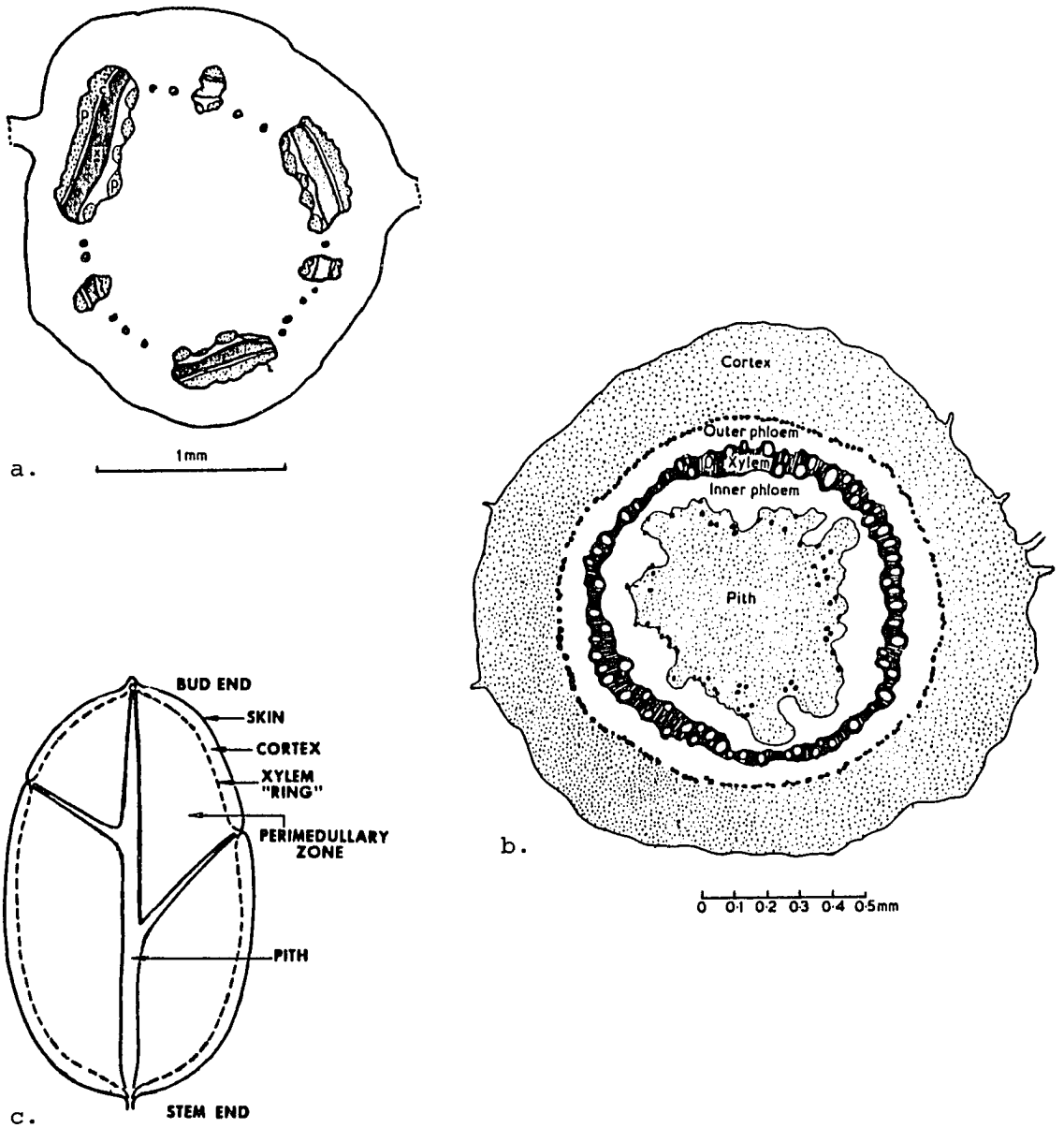



Figure 1. Diagrammatic views of potato stems, stolon, and tuber. -- a. Internodal region of young stem in primary growth. Three large and three smaller bicollateral vascular bundles are present (C, cambium; P, internal and outer phloem; x, xylem) (Cutter, 1978); b. Mature stolon showing relative areas occupied by various tissues (Artschwager, 1924); c. Mature tuber showing an idealized longitudinal diagram (Reeve, Hautala, and Weaver, 1969).

their enlargement is greatest in the perimedullary and cortex regions of the bud end and mid section, and least in the stem end and pith tissues (Reeve et al., 1969; Reeve et al., 1971). Chung and Hadziyev (1980) and Reeve et al. (1973) showed that starch grain sizes are larger in the perimedullary zone region than in the cortex or pith. The size of the starch grain is maximum at maturation (Reeve et al., 1973; Smith, 1968), but the size achieved depends on the variety and location (Smith, 1968).

## Environmental Effects on Top and Tuber Growth

### 1. Temperature and Light Effects

Marinus and Bodlaender (1975) concluded that the optimal temperature for growth of most potato cultivars is between 16°C and 25°C. However, studies relating temperature effects on potato development are limited and conflicting, probably because light also plays a role in development.

There is general accord on the temperature optimum for emergence. Bodlaender (1963) examined a number of European cultivars under greenhouse conditions and stated that shoot emergence increased as the air temperature was raised and emergence peaked at 22°C. Under field conditions in Australia, Sale (1979) found that emergence was optimal when the temperature was 22°C to 24°C and decreased sharply above 24°C.

Data about temperature effects on subsequent development, however, differ widely. Bodlaender (1963) noted that the optimum temperature for stem elongation was 18°C, and the temperature optimum for later

leaf development was an even lower 16°C. In greenhouse studies at 16°C, 22°C, and 27°C, Bodlaender noted that stem:leaf weight ratios increased, whereas tuber dry weights decreased with increasing temperatures. Further studies showed that an air temperature of 25°C favors stolon growth, whereas 20°C induced tuber initiation, and maximum tuber weights occurred when air and soil temperatures were between 18°C to 20°C. Moorby and Milthorpe (1975) obtained similar results and concluded that low night temperatures of 17°C tended to increase tuber numbers per plant. These trends are in accord with what might be expected for potatoes cultivated in the Andes, where they originated. In the Andes, potatoes are planted in the summer and harvested during the fall as temperatures begin to drop (Hawkes, 1978).

Bodlaender (1963) recognized some effects of light intensity on potato development and noted that intensities of 300 $\mu E\ m^{-2}\ sec^{-1}$ (about 1/5 full sunlight) resulted in heavier branching and higher leaf and tuber weights than in plants given 150 $\mu E\ m^{-2}\ sec^{-1}$, but leaves of plants grown at the higher light levels senesced earlier.

Studies conducted at even higher light intensities showed that light can greatly alter the temperature optimum for development of potatoes. Ku, Edwards, and Tanner (1977) noted that a potato plant's maximum physiological activity in both field and growth chamber experiments was reached when light intensities of about 850 $\mu E\ m^{-2}\ sec^{-1}$ were used. Benoit et al. (1983) varied the temperature regimes from 10°C to 35°C under 850 $\mu E\ m^{-2}\ sec^{-1}$ and noted that maximum leaf area increase occurred at 25°C, but leaf growth rates decreased slowly between 30°C and 35°C

and the rate of stem elongation was optimal at 30°C. Sale (1974) and Moorby (1970) also stated that top and tuber growth can be limited by the intensity and duration of light. Sale (1973a, 1973b, 1974) found that at full sunlight intensity of about 800 w $m^{-2}$, top and tuber yield was high even at 30°C, but shading to 200 w $m^{-2}$ at 30°C reduced yield considerably. It appears, therefore, that the high light intensities encountered in Arizona are a major factor in allowing high yields of potatoes, even though temperatures may exceed the stated optimum.

2. Water Status and Potato Plant Development

Water status values of potatoes as well as other plants are affected by the rate of transpiration, soil water availability, and density of roots (Moorby, Munns, and Walcott, 1975). de Lis, Ponce, and Tizio (1964) noted that water deficit effects were more detrimental during stolon growth and tuber initiation than during tuber growth, but Singh (1969) stated that high soil moisture levels should be maintained during all stages of growth for maximum tuber yield. Moorby and Milthorpe (1975) and Moorby et al. (1975) found that high temperatures during the bulking period could reduce leaf water potential values because transpiration rates can exceed water absorption rates in even well-watered soils. They stated that water stress for short periods stopped or retarded tuber growth, and relieving the stress did not lead to normal tuber development. Instead growth was restricted to specific regions of the tuber, and secondary growth led to development of irregularly shaped tubers of lower quality.

The immediate effect of water stress is stomate closure, which will lead to reduced transpiration, $CO_2$ gas exchange, and leaf elongation rates (Baker and Moorby, 1969; Moorby et al., 1975; Ackerson et al., 1977). However, Baker and Moorby, Moorby et al., and Ackerson et al. were unable to see changes in the level of sugars in leaves or distribution of sugars in stems and tubers. They were also unable to see changes in activity of starch synthesizing enzymes in tubers, and ribulose bisphosphate carboxylase (RUBP Case) and phosphoenolpyruvate carboxylase (PEPcase) activities in the leaves.

Gandar and Tanner (1976a, 1976b) concluded also from greenhouse and field experiments that leaf and tuber expansion is closely associated with transpiration rates and amounts of available soil water. They concluded that tuber weight increases and leaf elongation rates were higher at night because of higher water potential.

## Tuber Quality and Yield

A tuber's quality is determined by its appearance, flavor, and texture, and many of the factors affecting these properties are now understood.

The darkening of potatoes that occurs after cooking is related to the ratio of citric acid to chlorogenic acid, with low ratio leading to increased darkening (Smith, 1968).

The flavor and aroma of tubers are also affected by volatile compounds such as carbonyls, formaldehyde, acetaldehyde, acetone, methyl-ethyl ketone, and methyl-isopropyl ketone (Smith, 1968).

In the case of potato chips, the color show after frying is related to contents of starch, sucrose, and reducing sugars. Generally, tubers with high starch and low soluble sugars yield lighter colored chips and are considered desirable. In addition to causing darker chip colors, high soluble sugars also serve as a precursor for the formation of volatile ketones and other substances that affect flavor (Smith, 1968).

The contents of starch, sucrose, and reducing sugars are particularly important in tubers used for the chipping industry, and the levels of these substances can also be related to tuber maturity, specific gravity (SpG), and dry matter percentages (% DM). Generally, cultivars having tubers with high starch and low soluble sugars are considered good processing types (Sowokinos, 1971, 1973, 1976).

During early stages of tuber development free sugar contents are high, and as maturation proceeds starch percentages increase (Sowokinos, 1971). Because growing starch grains reduce the amount of intercellular space, SpG values and % DM are increased (Chung and Hadziyev, 1980; Reeve et al., 1969; Reeve et al., 1973). After Smith (1950) developed procedures for the industrial determination of SpG, several workers (e.g., Fitzpatrick, Porter, and Houghland, 1969; Kushman and Pope, 1968; Kushman, 1969) showed a direct relationship of SpG to % DM, but some differences were found in the regression equations showing this relationship. These differences may be due to cultivar types (Fitzpatrick et al., 1969; Kushman, 1969) or location of growth (Schwimmer and Burr, 1959). SpG values of potatoes grown in

southern regions of the United States are often lower than those grown in northern regions of the United States (Johansen et al., 1967). Environmental factors (Motes and Greig, 1970) and tuber size (Ifenkova, Allan, and Wurr, 1974) also affect SpG and % DM content. Despite the fact some varibility exists in different regions, SpG determinations have served as a convenient means for the grower to determine suitable maturing and to estimate starch content.

## Sucrose and Starch Metabolism during Tuber Growth and Storage

### 1. Sucrose Synthesis

The synthesis of sucrose requires sugar nucelotides (e.g., (ADP)-glucose) which can be formed by reacting nucleotide triphosphates with a hexose-1-phosphate by a pyrophosphorylase-mediated reaction (Espada, 1962) or by reaction of sucrose with a nucleotide diphosphate (e.g., UDP(ADP)) (Cardini, Leloir, and Chiriboga, 1955; LeLoir and Cardini, 1953, 1955). The enzyme which regulates the latter reaction, sucrose synthase, was originally considered the one involved in sucrose synthesis (Leloir and Cardini, 1955). More recent evidence, however, indicates sucrose is made via a 2-step reaction involving first a sucrose-P synthase controlled reaction of UDP(ADP)-glucose with fructose-6-P to yield sucrose-P and UDP(ADP) and a later phosphatase controlled hydrolysis of the sucrose-P to yield free sucrose and inorganic phosphate (Pi) (Feingold and Avigad, 1980). In leaves, where sucrose is largely formed and subsequently transferred to the phloem, the supply of the necessary cytoplasmic fructose-6-P needed for the

sucrose-P-synthase reaction is controlled by the activity of fructose 1,6-biphosphatase that in turn is affected by levels of fructose-2,6 $P_2$ (Herzog, Stitt, and Heldt, 1984). It is believed that when photosynthesis rates are very high, or by other means, levels of fructose-2,6 $P_2$ will rise and inhibit the formation of fructose-6-P (Herzog et al., 1984). This tends to cause accumulation of triose-P within the chloroplast and a slowdown in the exit of Pi from the chloroplast leading to increased starch synthesis (Heldt et al., 1977).

2. Starch Synthesis

Starch formation in developing tubers depends upon the supply of sucrose arriving by phloem transport and, except for possible light effects, it is believed that starch formation is regulated in a manner similar to that found in leaves (Preiss and Levi, 1980; Preiss, 1982). Starch formation occurs in amyloplasts of tubers, and the translocator molecules regulating entry of precursors are considered similar or the same to those of chloroplasts (Sowokinos, 1976, 1981). As in chloroplasts of leaves, ADP-glucose pyrophosphorylase catalyzes the reaction of glucose-1-P to ADP-glucose and starch synthases which transfer glucose from sugar nucleotides to the oligosaccharide primer (Preiss, 1982).

3. Interconversion and Breakdown of Sucrose and Starches in the Tuber

During tuber growth, starch grains also grow and amyloplast extrudes into the vacuolar space while still connected to the cytoplasm by a "stalk". As the starch grain grows, the surrounding plastid and

cytoplasmic matrix become thinner (Ohad et al., 1971). During tuber maturation the cytoplasmic matrix disappears, but starch grains are still enclosed within the intact plastic double membranes (Ohad et al., 1971). Storage at 4°C resulted in disappearance of plastid membranes, and reappearance of these membranes occurred around the starch grains when the storage temperature shifted to 25°C (Ohad et al., 1971).

Isherwood (1973, 1976) summarized changes between starch and sucrose interconversion during cold storage. He stated that below 5°C storage causes soluble sugars to accumulate rapidly and steady-state levels are achieved within a few weeks; he termed this "low-temperature sweetening". When tubers are returned to moderate temperatues (10°C) after short duration storage, soluble sugars decline and starch is almost quantitatively reformed (Isherwood, 1973). Storage of tubers for several months leads to an increase in soluble sugars and irreversible aging. Isherwood (1976) called this "senescent sweetening", and it is related to disintegration and repair of the plastid membranes.

A noticeable increase in the level of sucrose with a concomitant reduction of starch occurs during cold storage of potato tubers. This sucrose is further hydrolyzed by invertase (Pressey and Shaw, 1966) during cold storage. Pressey and Shaw also showed increases in invertase inhibitor activity at 18°C lowered the amount of reducing sugars. ap Rees (1980) stated that invertases have several isozymes and are mostly localized in the vacuoles and apoplasm of the cells.

Both α-amylase and starch phosphorylase are present at relatively high amounts during maturation and these activities are unchanged

during storage, but levels of both increase slightly during sprouting (Bailey, Phillips, and Pitt, 1978). Hydrolysis of amylase by α-amylase and degradation of amylopectin by potato phosphorylases as well as the debranching enzymes (R-enzyme), transglycosylase (D-enzyme), and β-amylase are responsible for hydrolyzing the starch in situ (Preiss, and Levi, 1980). However, the mechanism of their regulation during storage and their relationship to glycolitic intermediates in plastids or in the cytoplasm are not yet known (Preiss, 1982; ap Rees, 1980).

## Materials and Methods

In 1979, studies were conducted at the University of Arizona Experiment Station in Mesa, Arizona and at the Campbell Avenue University Farm in Tucson, Arizona. Seed pieces of the cultivars 'Kennebec', 'Norgold Russet', 'Denali', 'Red LaSoda', 'Nebraska 118', and 'Nebraska S1-3' were planted in Mesa on February 9, 1979, and tubers were harvested on June 7, 1979. These were part of the Advanced Clone Potato Trials conducted by Drs. R. O'Keeffe and P. M. Bessey. In Tucson, the cultivars 'Kennebec', 'Norgold Russet', 'Denali', 'Red LaSoda', and 'Nebraska S1-3' were planted on March 3, 1979 and harvested on July 5, 1979.

The 1980 studies were conducted in Mesa and at the Marana University Experiment Station at Marana, Arizona. The cultivars 'Kennebec', 'Norgold Russet', and 'Denali' were used in both locations. At Mesa, seed pieces were planted on March 5, and tubers and plants were harvested on July 2; at Marana, planting was on March 21 and harvests were made on July 16.

The soils used in the field studies were silty loams, and the climatic conditions encountered are provided in Figure 2 and in Tables 1 and 2.

Field preparations, leaf area index (LAI) determinations, plant sampling, and preparation and extraction of samples with 80% ethanol were performed as described by Arslan et al. (1985). Additionally, cultivars from the 1979 harvest in Mesa were also stored at 9°C for 1 year and tubers were analyzed for sucrose, glucose, fructose, total soluble sugars (TSS), and starch (Arslan et al., 1985) immediately following cold storage and also 12 days after holding at room temperature.

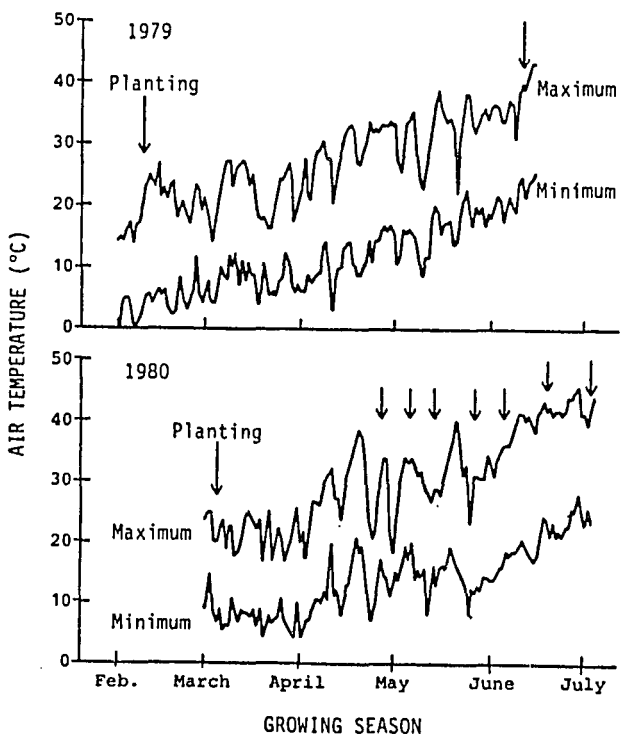



Figure 2. Daily maximum and minimum temperatures during 1979 and 1980 at Mesa. -- Source: Anonymous (1979, 1980)

Table 1. Monthly climatic data at the Campbell Avenue Experimental Farm, Tucson, Arizona, during 1979[a]

| Month | Precipitation (mm) | Evaporation (mm) | Mean Relative Humidity[b] (%) | Mean Temperature Day (°C) | Mean Temperature Night (°C) |
|---|---|---|---|---|---|
| February | 21 | 122 | 33 | 20.4 | 1.9 |
| March | 23 | 155 | 32 | 21.8 | 7.8 |
| April | 0 | 279 | 15 | 29.0 | 7.1 |
| May | 13 | 326 | 20 | 31.7 | 12.4 |
| June | 4 | 401 | 16 | 37.9 | 18.2 |
| July | 31 | 413 | 25 | 39.8 | 20.8 |

a. Climatic data were obtained with standard U.S. Weather Bureau instrumentation within 500 m from the field site.

b. Mean monthly relative humidities were measured between 11:00 and 17:00 hr.

Source: Anonymous (1979, 1980).

Table 2. Monthly climatic data at the Mesa Experimental Farm, Mesa, Arizona, during 1979 and 1980[a]

| Month | Precipitation (mm) | Evaporation (mm) | Mean Relative Humidity[b] (%) | Mean Temperature Day (°C) | Mean Temperature Night (°C) |
|---|---|---|---|---|---|
| | | 1979 | | | |
| March | 51 | 152 | 36 | 22.0 | 8.1 |
| April | 0 | 243 | 17 | 29.0 | 11.2 |
| May | 13 | 303 | 20 | 32.2 | 16.4 |
| June | 1 | 361 | 13 | 39.1 | 21.4 |
| July | 14 | 392 | 21 | 41.5 | 24.6 |
| | | 1980 | | | |
| March | 26 | 147 | 34 | 21.6 | 7.8 |
| April | 9 | 241 | 22 | 28.2 | 12.0 |
| May | 19 | 312 | 18 | 30.8 | 14.6 |
| June | 0 | 377 | 10 | 39.8 | 20.2 |
| July | 6 | 385 | 22 | 42.0 | 25.7 |

a. Climatic data were obtained with standard U.S. Weather Bureau instrumentation within 500 m from the field site.

b. Mean monthly relative humdities were measured between 11:00 and 17:00 hr.

Source: Anonymous (1979, 1980).

Leaf temperatures and the ambient temperature over the canopy and water potentials of the cultivars growing on well-watered soils were determined at intervals from sunrise to sundown on a hot and cloudless day in both Mesa (June 19, 1980; 107 days after planting) and Marana (June 29, 1980; 97 days after planting) during rapid tuber growth.

Leaf temperatures of the upper, middle, and lower leaves, soil temperatures about 5 cm below the ground surface underneath the canopy, and ambient temperatures about 1 m above the canopy were measured with a Bailey BAT-12 (Bailey Ins. Incorporated) needle-point thermocouple digital thermometer. Leaf temperatures were obtained by insertion of the needle into the veins of the leaves.

Measurements of water potentials of upper, middle, and lower leaves were obtained with a pressure chamber (PMS, Model 600 psi) according to Scholander et al. (1965).

## Measurements of Tuber Quality Characteristics of the Cultivars

### 1. Physiological Yield and Marketable Yield

Total yield values of cultivars at harvest were determined using the average values of tuber yield per plant which was extrapolated to 40,000 plants per hectare. Marketable yield was represented values obtained with tubers that were 100 g or larger in weight (Arslan et al., 1985).

2. Dry Matter Content of the Tubers

Dry matter content (% DM) of the tubers was measured during tuber growth and at final harvest. After tuber fresh weights (FW) were taken and samplings for carbohydrate analysis were performed, the remaining tubers were sliced further, placed in paper bags, and heated initially to 100°C. Temperature settings were gradually reduced, and after tuber temperature approached 60°C the oven settings were set at 60°C until complete drying. Tuber dry weights (DW) obtained this way were identical to DW of tuber tissues obtained by lyophilization. Corrections were made for tubers to express % DM as percentage of FW.

3. Specific Gravity Determination

Specific gravity (SpG) was determined according to the procedure developed by Smith (1950).

4. Chipping Quality Determination

A frying procedure developed by O'Keeffe at the University of Nebraska was used. The color of the mini chips after frying was visually compared with the McLaughlin Standard Chart provided by the Potato Chip International Institute (now PC/SFA, Potato Chip/Snack Food Association).

## Results and Discussion

### Duration from Planting to Emergence, Flowering, and Harvesting of Potatoes

According to Pew et al. (1976), potato seed pieces should be planted no earlier than 1 week following the last anticipated frost

date, but the planting dates may require change because of unexpected rain, etc. Because prevailing temperature conditions as well as locale and other factors can potentially affect development and yield, the durations required to reach specific stages of development were noted in several of the studies.

In 1979, specific information relating planting date to developmental stage in Mesa was not obtained, but seed pieces were planted on February 9 and tubers were harvested after 4 month's growth (June 7). In Tucson, seed pieces were planted about 1 month later (March 3), and emergence of all cultivars occurred in about 3 weeks (March 25). Most cultivars flowered by 53 days (April 24) and all cultivars showed heavy branching after 65 days (May 6). Tubers were also harvested 4 months after planting (July 5). Although some differences were noted among the cultivars in the times required to reach a specific developmental stage, the differences were not pronounced.

A similar time frame was involved in the 1980 studies where seed pieces were planted in Mesa on March 5 and tubers were harvested on July 2. Flowering of all cultivars occurred after 45 days' growth, and heavy stem development was evident by 52 days (April 26). The somewhat shorter time period needed to reach flowering and branch development during 1980 may be a reflection of temperature differences existing between Mesa (Fig. 2; Table 2) and Tucson (Table 1). Temperature data were not available for Marana during the 1980 growing season, but plantings and harvest were later (March 21 and July 15, respectively) than in Mesa.

Relationship of Leaf Area to Leaf Length and Leaf Disc Dry Weight

As Epstein and Robinson (1965) concluded that leaf areas of potatoes are directly related to leaf length, the possible existence of this relationship for plants grown in Arizona was investigated in Tucson during 1979. Studies with 5 cultivars grown in the greenhouse clearly showed that areas can be correlated with leaf length (Fig. 3). A similar relationship was found for leaves derived from the primary stem in field-grown plants, and measurements of leaf length showed that

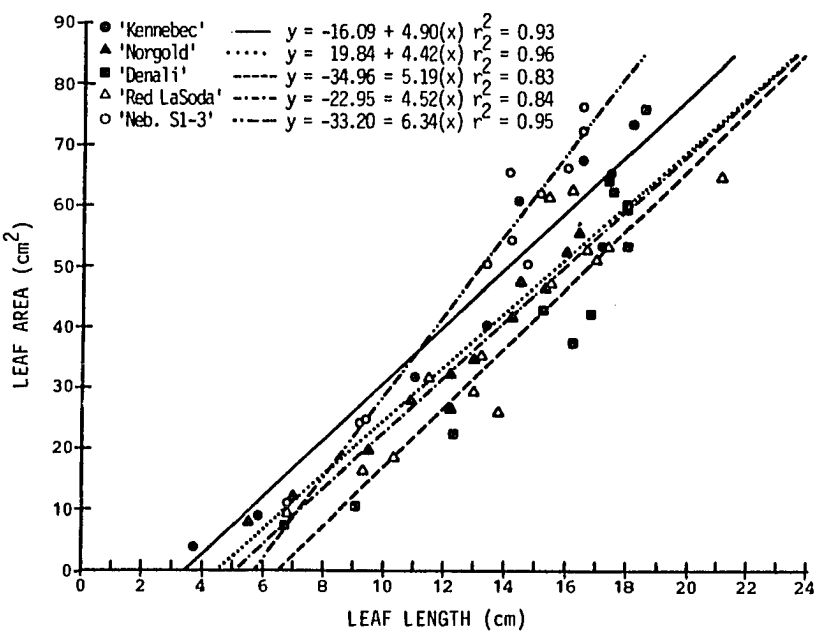



Figure 3. The relationship between leaf length and leaf area of greenhouse-grown potato cultivars. -- The 1979 greenhouse studies were conducted with a composite soil mixture consisting of 1 part of a sandy loam soil from the University of Arizona Casa Grande Overpass Farm, 1 part cow manure, and 1 part sand, and the mixture was then steam sterilized for 2.5 hr. A single seed piece was placed in a metal can which held 4 L soil. Leaf areas were obtained using a Hayashi Denko Model AAM5 optical leaf area analyzer. The values were the average values obtained from 12 plants for each cultivar.

elongation occurred for more than 25 days, but lengths often decreased later because of desiccation from the tip downward. Most of the cultivars reached to 20 to 22 cm in length, but growth of 'Nebraska S1-3' was less; however, because leaf areas per length were greater for this cultivar than the others (Fig. 4), leaf areas of this cultivar were comparable to the others.

Leaves derived from the primary stems showed a high correlation of length to area, but visual examination of leaves derived from axillary branches following flowering showed wide variation in leaf morphology, and attempts to correlate length to area yielded $r^2$ values that were usually about 0.4. As a consequence, an alternative procedure was sought for determining leaf areas.

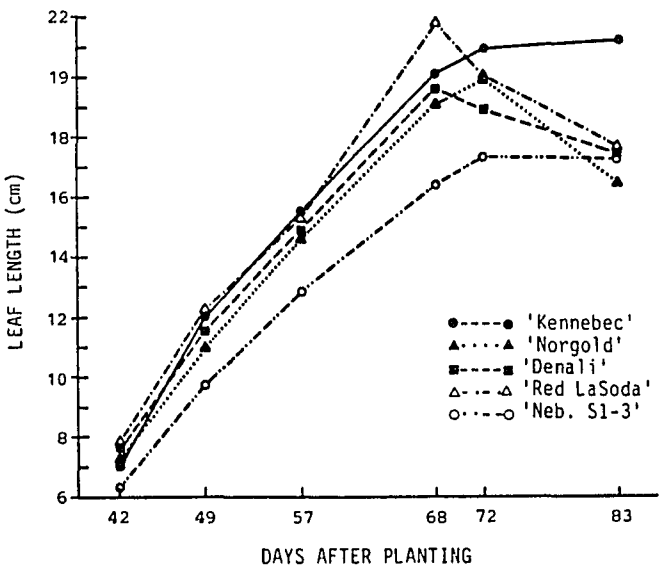



Figure 4. Leaf lengths of potato cultivars grown at the Campbell Avenue Experimental Farm in 1979. -- After field emergence, third leaf from the tip of the main stem was tagged. Average values represent 12 such labelled plants' leaf length growth for each cultivar.

In 1980, areas were determined by comparing DW of a 12 mm disc with weights for the whole leaf (Arslan et al., 1985; Sale, 1979). Seven consecutive biweekly samplings from 3 plots for each cultivar in Mesa and 4 consecutive samplings in 3 plots for each cultivar in Marana showed that 25 leaf disc DW (12 mm in diameter) were nearly constant (0.25 g/25 disc) regardless of cultivars. Although 25 disc dry weights in Mesa (0.24 g/25 disc) were slightly lower than Marana (0.26 g/25 disc), the results showed that leaf areas can be determined by comparing total leaf weights to disc weights.

Relationship of Top to Tuber Growth

Although the 1979 study at the Campbell Avenue Experiment Station Farm in Tucson was designed to study the relationship of top to tuber growth, the field was later found to have a water impermeable layer. Because this led to waterlogging, poor stands, low growth rates, and disease problems, measurements of top growth and tuber development were made only at final harvest. Top to tuber comparisons were also conducted at the Mesa Agricultural Experiment Station in 1979, but plantings were late (March 17), and final harvests were made after a short growth period. As a consequence, the 1979 studies provided limited information.

At final harvest in Tucson, tops showed considerable desiccation and senescence. Tubers were found on all plants, but only a fraction were marketable (Table 3). Fresh weight yields from the cultivars 'Red LaSoda' and 'Norgold Russet' were lower than those of the other cultivars.

Table 3. Top and tuber properties of potato cultivars grown at the Campbell Avenue Experimental Farm, Tucson, Arizona, at final harvest. -- Harvest was on July 5, 1979, and data represent mean ± SD values from 12 plants for each cultivar.

| Potato Cultivar | Top | | Tubers | | | Marketable Tubers by Weight Classes | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 100-200 g | | > 200 g | |
| | Fresh Weight | Dry Weight | Fresh Weight | Dry Weight | Number Tubers | Number | Mean Fresh Weight | Number | Mean Fresh Weight |
| 'Denali' | 109 ± 30 | 48 ± 14 | 412 ± 18 | 83 ± 6 | 13 ± 5 | 2.0 | 134 ± 23 | 0.0 | 0.0 |
| 'Kennebec' | 76 ± 21 | 30 ± 1 | 375 ± 83 | 73 ± 13 | 8 ± 2 | 1.6 | 125 ± 21 | 0.4 | 256 ± 33 |
| 'Red LaSoda' | 51 ± 38 | 21 ± 13 | 298 ± 69 | 58 ± 14 | 9 ± 4 | 1.1 | 127 ± 27 | 0.1 | 221 ± 4 |
| 'Nebraska S1-3' | 123 ± 50 | 42 ± 18 | 379 ± 118 | 69 ± 23 | 19 ± 4 | 0.4 | 121 ± 21 | 0.0 | 0.0 |
| 'Norgold Russet' | 41 ± 29 | 21 ± 11 | 246 ± 143 | 46 ± 27 | 7 ± 2 | 0.5 | 123 ± 26 | 0.0 | 0.0 |

Measurements of plant height over time of plants grown in Tucson suggested a triphasic growth curve (Fig. 5) with a rapid and linear height increase of about 3 weeks' duration beginning from April 15. The reduced growth that occurred 64 days after planting (May 7) appeared to coincide with the onset of extensive branching. However, quantitative determination of branching was not made.

In Mesa during 1979, tubers were present by 66 days after planting (Table 4). Tubers of marketable size were obtained on this date and numbers of marketable tubers did not increase significantly at final harvest. Although tuber numbers were generally lower than those in Tucson (Table 3), tubers were larger.

Plants in Mesa showed little to no evidence of desiccation or senescence, and at final harvest, DW of the Mesa-grown tops were usually about 20% of FW as compared to about 40% in Tucson (calculated from Tables 3 and 4). Interestingly, % DM content of tubers of 'Norgold Russet' and 'Red LaSoda' at nearly all harvest periods was slightly lower than the approximately 20% values found for 'Nebraska 118', 'Denali', and 'Kennebec'.

In both Tucson and Mesa (Tables 3 and 4), the trial clones from Nebraska ('Neb. S1-3' and 'Neb. 118') showed superior height response. Generally, plants in Mesa were taller than those in Tucson. Although plant height is considered an indication of growth (Benoit et al., 1983), the results in Tucson and Mesa did not show any clear relationship of height to tuber weight.

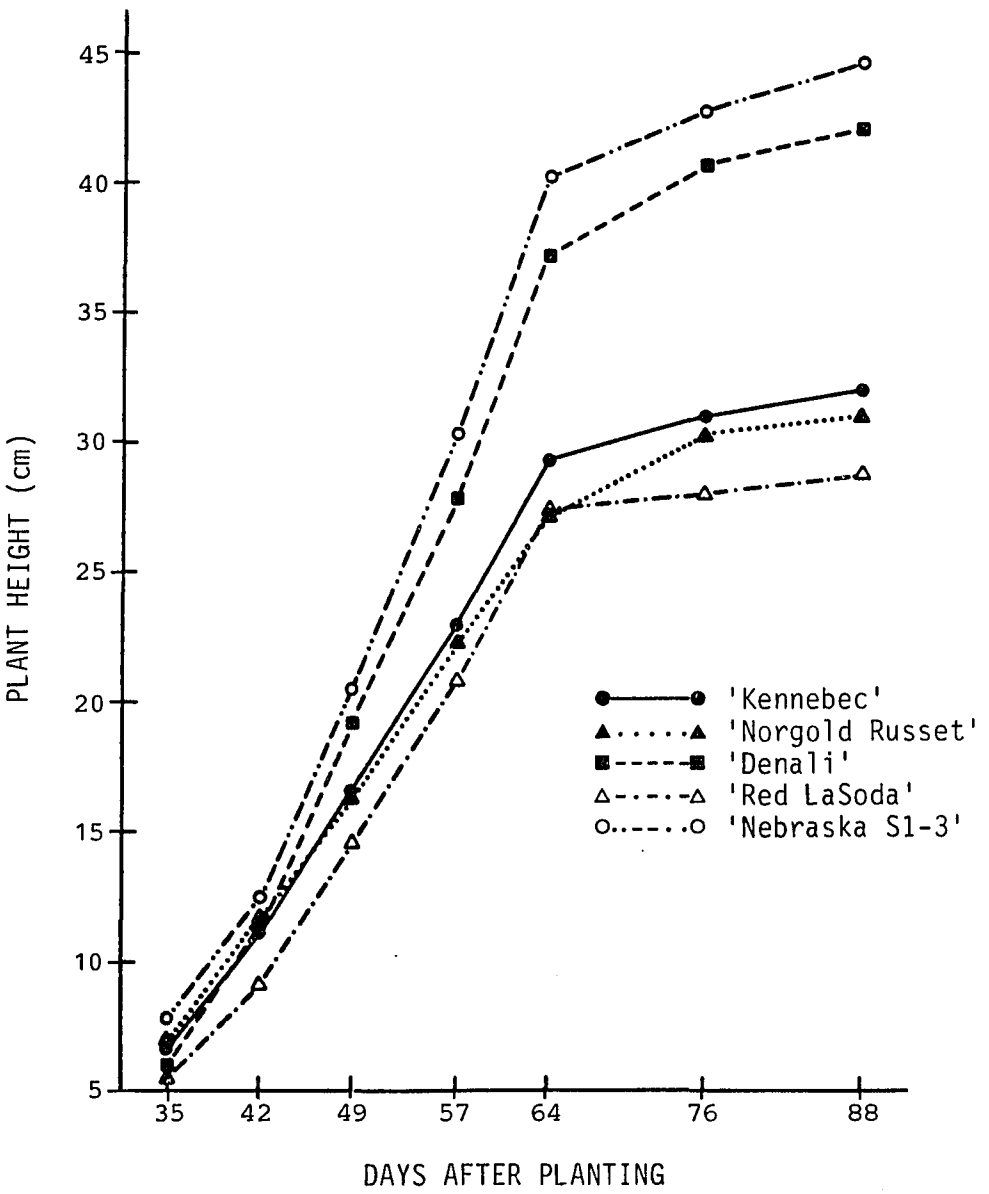



Figure 5. Changes in plant height of cultivars grown in Tucson, 1979. -- Values represent averages from 12 plants for each cultivar.

Table 4. Top and tuber growth of potato cultivars grown at the Mesa Experimental Farm, Mesa, Arizona, during 1979. -- Values represent mean ± SD for 4 plants for each cultivar.

| Potato Cultivar | Top | | | Tuber | | | |
|---|---|---|---|---|---|---|---|
| | Fresh Weight (g) | Dry Weight (g) | Plant Height (cm) | Fresh Weight (g) | Dry Weight (g) | Mean Number Tubers[a] | Marketability (%)[b] |
| | | | May 21 (66 days after planting) | | | | |
| 'Norgold' | 90 ± 54 | 13 ± 8 | 24 ± 9 | 143 ± 149 | 24 ± 27 | 3.0 ± 3.4 ( 2) | 0.17 |
| 'Red LaSoda' | 228 ± 131 | 30 ± 18 | 36 ± 2 | 403 ± 259 | 60 ± 42 | 5.0 ± 4.0 ( 4) | 0.20 |
| 'Nebraska 118' | 289 ± 56 | 46 ± 6 | 48 ± 9 | 315 ± 152 | 60 ± 30 | 2.3 ± 2.6 ( 6) | 0.60 |
| 'Denali' | 143 ± 47 | 24 ± 4 | 35 ± 5 | 381 ± 86 | 77 ± 16 | 4.3 ± 2.1 ( 4) | 0.24 |
| 'Kennebec' | 227 ± 83 | 31 ± 11 | 40 ± 11 | 364 ± 141 | 76 ± 30 | 2.0 ± 1.4 ( 4) | 0.50 |
| | | | June 1 (76 days after planting) | | | | |
| 'Norgold' | 137 ± 78 | 22 ± 10 | 29 ± 7 | 428 ± 83 | 73 ± 18 | 4.8 ± 3.0 ( 5) | 0.25 |
| 'Red LaSoda' | 292 ± 131 | 38 ± 13 | 37 ± 8 | 444 ± 305 | 74 ± 51 | 4.5 ± 1.3 ( 7) | 0.39 |
| 'Nebraska 118' | 311 ± 89 | 46 ± 12 | 56 ± 8 | 490 ± 165 | 101 ± 30 | 5.5 ± 1.9 ( 8) | 0.36 |
| 'Denali' | 222 ± 101 | 36 ± 14 | 38 ± 6 | 516 ± 202 | 110 ± 41 | 6.3 ± 4.6 ( 8) | 0.32 |
| 'Kennebec' | 312 ± 222 | 43 ± 27 | 37 ± 7 | 516 ± 286 | 94 ± 56 | 3.3 ± 2.6 ( 9) | 0.60 |
| | | | June 5 (80 days after planting) | | | | |
| 'Norgold' | 98 ± 113 | 16 ± 14 | 31 ± 10 | 29 ± 21 | 31 ± 10 | 1.8 ± 1.0 ( 3) | 0.14 |
| 'Red LaSoda' | 278 ± 25 | 40 ± 7 | 35 ± 21 | 83 ± 13 | 35 ± 21 | 5.0 ± 1.4 ( 4) | 0.20 |
| 'Nebraska 118' | 384 ± 55 | 52 ± 6 | 63 ± 14 | 106 ± 26 | 63 ± 14 | 5.3 ± 1.0 ( 8) | 0.24 |
| 'Denali' | 295 ± 143 | 34 ± 13 | 44 ± 9 | 110 ± 40 | 44 ± 9 | 5.8 ± 3.5 (10) | 0.13 |
| 'Kennebec' | 280 ± 71 | 36 ± 8 | 44 ± 6 | 95 ± 37 | 44 ± 6 | 3.8 ± 2.1 ( 8) | 0.31 |
| | | | June 8 (83 days after planting) | | | | |
| 'Norgold' | 88 ± 40 | 15 ± 6 | 21 ± 5 | 59 ± 34 | 21 ± 55 | 4.5 ± 1.9 ( 6) | 0.00 |
| 'Red LaSoda' | 173 ± 47 | 27 ± 7 | 45 ± 10 | 68 ± 16 | 45 ± 10 | 3.8 ± 1.3 ( 7) | 0.18 |
| 'Nebraska 118' | 224 ± 44 | 38 ± 10 | 55 ± 13 | 68 ± 48 | 55 ± 13 | 3.5 ± 0.6 ( 5) | 0.14 |
| 'Denali' | 135 ± 40 | 27 ± 6 | 43 ± 4 | 93 ± 27 | 43 ± 4 | 4.3 ± 0.5 ( 7) | 0.24 |
| 'Kennebec' | 402 ± 31 | 55 ± 2 | 50 ± 6 | 127 ± 56 | 50 ± 6 | 4.3 ± 1.0 ( 9) | 0.24 |

a. Numbers in parentheses indicate total number of marketable tubers from 4 plants.

b. Reduction in percentage of marketability after June 5 was due to hollow heart and glassy tubers.

In agreement with data found elsewhere (Sowokinos, 1971, 1973), analysis of the carbohydrate content of tubers at different times showed that TSS levels dropped and starch increased with tuber development (Table 5). However, the tubers from 'Norgold Russet' and "Red LaSoda' had higher TSS and lower starch levels than 'Nebraska 118', 'Denali', and 'Kennebec'.

Potato Top and Tuber Development
(Mesa and Marana, 1980)

In order to understand the possible basis for yield differences among cultivars, tuber yields obtained in Mesa and Marana at various times during 1980 were compared to vegetative growth characteristics. During early development in Mesa (up to 85 days), tuber yields of 'Kennebec' (Fig. 6), 'Norgold Russet' (Fig. 7), and 'Denali' (Fig. 8) were alike; however, yields of 'Kennebec' after about 85 days were clearly greater than those of 'Norgold Russet' or 'Denali'. These results contrast with those from Marana, where yields from all cultivars were similar throughout the growing season. At final harvest, tuber yields from all cultivars were lower than those obtained in Mesa; this may reflect the fact that seed pieces were planted on March 21, which was 16 days later than in Mesa. Pew et al. (1976) stated that in warmer areas of Arizona the ideal planting dates are between January 15 and February 15. They concluded later plantings prevented proper maturation of tubers, and temperature increases that occur in the middle of June and early July adversely affect tuber yield and quality.

Table 5. Carbohydrate composition of tubers of potato cultivars grown at the Mesa Experimental Farm, Mesa, Arizona, during 1979. -- Values for TSS and starch are mean ± SD for 3 determinations, expressed in percent dry weight.

| Compound | Potato Cultivar 'Norgold' (%) | 'Red LaSoda' (%) | 'Nebraska 118' (%) | 'Denali' (%) | 'Kennebec' (%) |
|---|---|---|---|---|---|
| | | | May 21, 1979 | | |
| TSS | 13.06 ± 0.35 | 7.66 ± 0.12 | 4.09 ± 0.20 | 2.41 ± 0.13 | 2.31 ± 0.28 |
| Starch | 40.78 ± 2.40 | 43.69 ± 6.60 | 51.48 ± 8.70 | 55.84 ± 4.90 | 50.14 ± 3.60 |
| | | | June 1, 1979 | | |
| TSS | 4.76 ± 0.43 | 3.19 ± 0.44 | 1.74 ± 0.10 | 1.66 ± 0.15 | 2.55 ± 0.37 |
| Starch | 52.92 ± 0.90 | 41.62 ± 2.23 | 57.55 ± 4.92 | 61.22 ± 0.95 | 58.67 ± 3.79 |
| | | | June 5, 1979 | | |
| TSS | 2.72 ± 0.20 | 2.32 ± 0.15 | 1.29 ± 0.11 | 1.05 ± 0.09 | 1.03 ± 0.15 |
| Starch | 55.11 ± 3.20 | 53.10 ± 2.54 | 61.88 ± 1.60 | 58.87 ± 1.70 | 60.46 ± 4.20 |
| | | | June 8, 1979 | | |
| TSS | 4.52 ± 0.50 | 2.23 ± 0.28 | 1.53 ± 0.24 | 0.88 ± 0.24 | 2.49 ± 0.34 |
| Starch | 54.86 ± 4.73 | 54.21 ± 0.93 | 60.82 ± 7.54 | 62.57 ± 5.22 | 62.45 ± 7.14 |

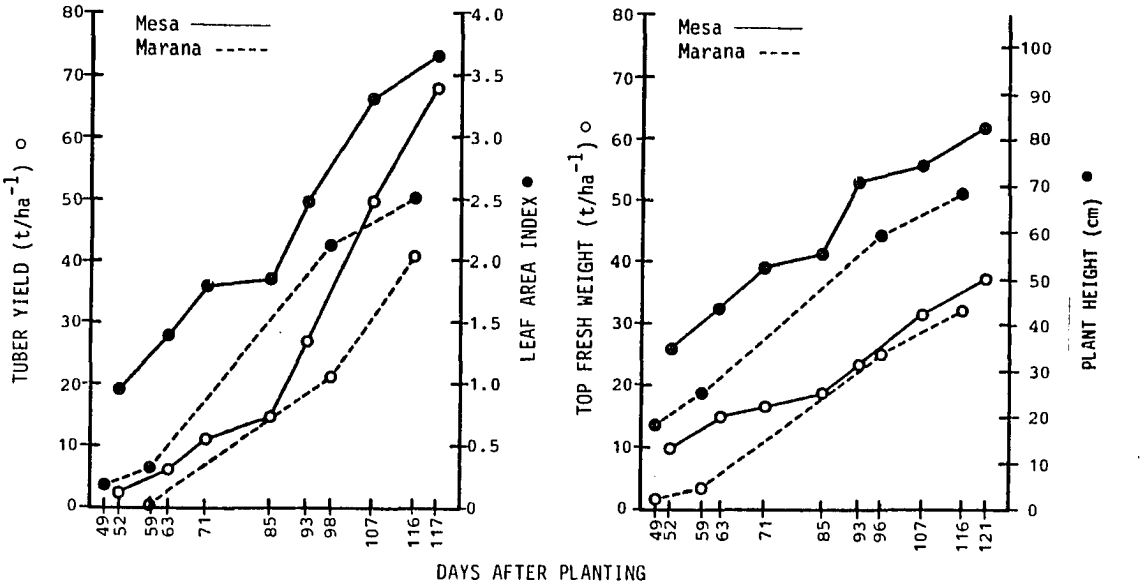



Figure 6. Time course changes in leaf area index, height, and top and tuber yields of 'Kennebec' plants grown in 1980. -- Solid lines represent average values obtained at Mesa. Dashed lines represent average values obtained at Marana.

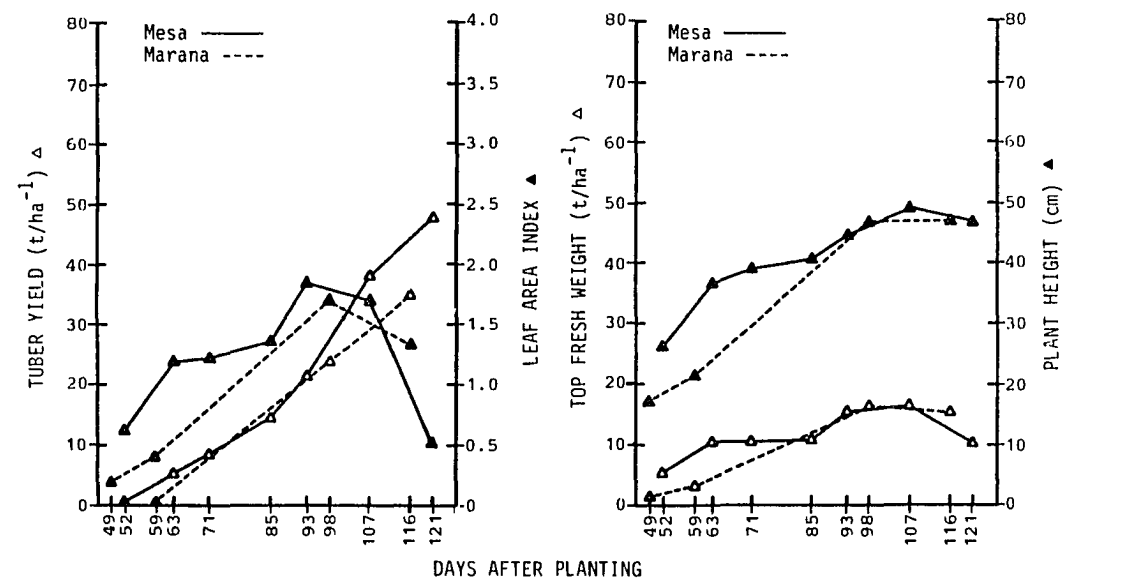



Table 7. Time course changes in leaf area index, height, and top and tuber yields of 'Norgold Russet' plants grown in 1980. -- Solid lines represent average values obtained at Mesa. Dashed lines represent average values obtained at Marana.

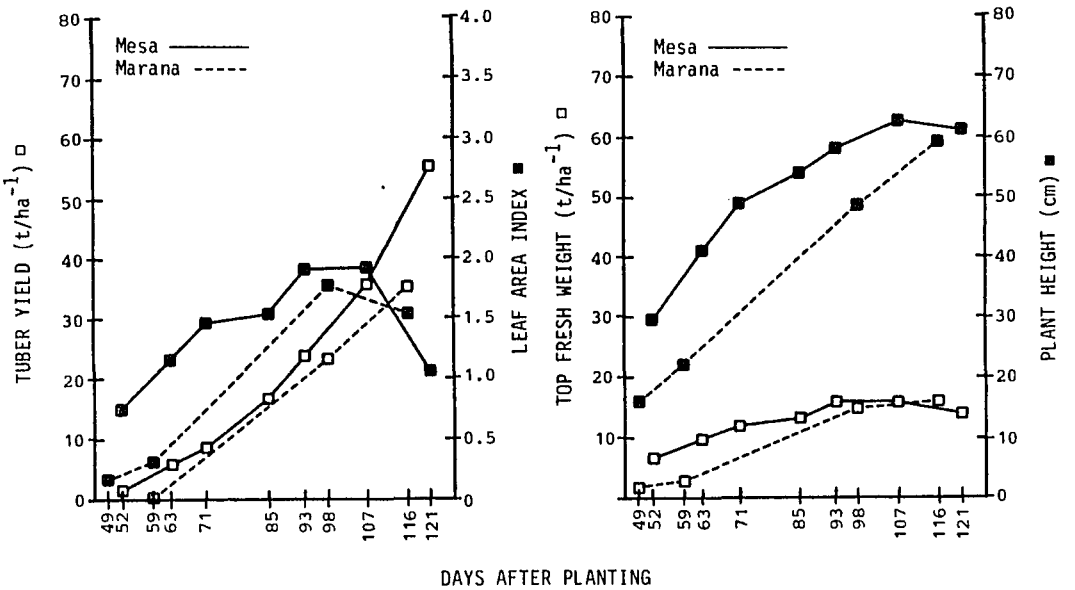



Figure 8. Time course changes in leaf area index, height, and top and tuber yields of 'Denali' plants grown in 1980. -- Solid lines represent average values obtained at Mesa. Dashed lines represent average values obtained at Marana.

As final tuber weights are far greater than the weights of leaves or stems (Tables 6, 7, and 8) and tuber production depends on on-going photosynthesis and assimilate availability, no attempt was made to directly relate tuber yield to either leaf or stem weight. Nevertheless, it should be noted that top FW of 'Kennebec' (Table 6) were greater than either 'Norgold Russet' (Table 7) or 'Denali' (Table 8) at final harvest. Because LAI values are a potential indicator of photosynthetic capacity, it is reasonable to link differences in tuber yield to possible differences in photosynthetic capacity as suggested by LAI values.

Table 6. Fresh and dry weights of leaves and stems of the potato cultivar 'Kennebec' grown at the Mesa and Marana Experimental Farms, Arizona. -- Values represent mean ± SD for 7 plants from 3 plots at each farm at each harvest.

| Days after Planting | Leaf | | Stem | |
|---|---|---|---|---|
| | Fresh Weight (g/plant) | Dry Weight (g/plant) | Fresh Weight (g/plant) | Dry Weight (g/plant) |
| | | Mesa, 1980 | | |
| 52 | 115 ± 7 | 13 ± 0.7 | 132 ± 4 | 11 ± 0.4 |
| 63 | 170 ± 45 | 18 ± 4.3 | 206 ± 67 | 17 ± 4.0 |
| 71 | 219 ± 23 | 22 ± 1.4 | 197 ± 37 | 21 ± 1.6 |
| 85 | 224 ± 12 | 27 ± 1.3 | 246 ± 17 | 28 ± 1.8 |
| 93 | 279 ± 22 | 35 ± 3.5 | 292 ± 37 | 34 ± 4.0 |
| 107 | 396 ± 73 | 54 ± 10.0 | 384 ± 26 | 46 ± 8.0 |
| 121 | 441 ± 85 | 56 ± 8.0 | 468 ± 58 | 54 ± 8.0 |
| | | Marana, 1980 | | |
| 49 | 24 ± 6 | 2 ± 1.3 | 19 ± 8 | 2 ± 0.7 |
| 59 | 42 ± 7 | 5 ± 1.0 | 41 ± 8 | 4 ± 0.6 |
| 98 | 274 ± 37 | 30 ± 2.5 | 355 ± 72 | 35 ± 6.0 |
| 116 | 321 ± 88 | 33 ± 4.0 | 481 ± 162 | 44 ± 11.0 |

Table 7. Fresh and dry weights of leaves and stems of the potato cultivar 'Norgold Russet' grown at the Mesa and Marana Experimental Farms, Arizona. -- Values represent mean ± SD for 7 plants from 3 plots at each farm at each harvest.

| Days after Planting | Leaf | | Stem | |
|---|---|---|---|---|
| | Fresh Weight (g/plant) | Dry Weight (g/plant) | Fresh Weight (g/plant) | Dry Weight (g/plant) |
| | | Mesa, 1980 | | |
| 52 | 75 ± 9 | 9 ± 1.0 | 67 ± 7 | 5 ± 0.6 |
| 63 | 142 ± 19 | 13 ± 2.3 | 132 ± 2 | 10 ± 1.8 |
| 71 | 147 ± 51 | 16 ± 2.2 | 114 ± 27 | 12 ± 2.1 |
| 85 | 160 ± 10 | 19 ± 1.3 | 117 ± 16 | 13 ± 2.0 |
| 93 | 214 ± 29 | 26 ± 5.3 | 170 ± 34 | 20 ± 7.3 |
| 107 | 197 ± 98 | 36 ± 5.6 | 220 ± 28 | 26 ± 1.5 |
| 121 | 66 ± 40 | 6 ± 0.1 | 206 ± 21 | 26 ± 2.9 |
| | | Marana, 1980 | | |
| 49 | 24 ± 1 | 2 ± 0.2 | 20 ± 2 | 2 ± 0.2 |
| 59 | 49 ± 7 | 6 ± 1.0 | 39 ± 6 | 4 ± 0.7 |
| 98 | 221 ± 50 | 23 ± 3.4 | 197 ± 76 | 20 ± 8.0 |
| 116 | 172 ± 58 | 17 ± 6.4 | 213 ± 68 | 21 ± 7.3 |

Table 8. Fresh and dry weights of leaves and stems of the potato cultivar 'Denali' grown at the Mesa and Marana Experimental Farms, Arizona. -- Values represent mean ± SD for 7 plants from 3 plots at each farm at each harvest.

| Days after Planting | Leaf | | Stem | |
|---|---|---|---|---|
| | Fresh Weight (g/plant) | Dry Weight (g/plant) | Fresh Weight (g/plant) | Dry Weight (g/plant) |
| | | Mesa, 1980 | | |
| 52 | 85 ± 12 | 11 ± 0.6 | 72 ± 11 | 6 ± 1.1 |
| 63 | 132 ± 14 | 16 ± 3.4 | 110 ± 8 | 11 ± 1.1 |
| 71 | 167 ± 11 | 22 ± 2.8 | 125 ± 15 | 15 ± 1.0 |
| 85 | 174 ± 29 | 23 ± 0.9 | 142 ± 10 | 16 ± 0.8 |
| 93 | 216 ± 67 | 28 ± 7.0 | 169 ± 55 | 19 ± 5.3 |
| 107 | 216 ± 108 | 38 ± 0.8 | 220 ± 17 | 25 ± 1.0 |
| 121 | 184 ± 69 | 17 ± 7.1 | 219 ± 24 | 25 ± 2.8 |
| | | Marana, 1980 | | |
| 49 | 21 ± 3 | 2 ± 0.1 | 14 ± 1 | 2 ± 0.2 |
| 59 | 38 ± 11 | 5 ± 1.6 | 27 ± 11 | 3 ± 1.1 |
| 98 | 211 ± 63 | 22 ± 4.8 | 155 ± 42 | 19 ± 5.1 |
| 116 | 183 ± 42 | 22 ± 6.6 | 208 ± 64 | 25 ± 7.0 |

Experiments at both Mesa and Marana showed that LAI values of 'Kennebec' increased continuously until final harvest, but LAI values for 'Denali' and 'Norgold Russet' either peaked or stabilized at 85-90 days after planting (Figs. 6, 7, and 8). This indicates that tuber growth during the late stage of development in 'Norgold Russet' and 'Denali' were occurring as photosynthetic capacity was declining and that high yields of 'Kennebec' obtained in Mesa (Fig. 6) may occur because this cultivar continued to increase in photosynthetic capacity. Additionally, LAI values of each of the cultivars tended to be higher in Mesa than in Marana during the first 70 to 80 days of development. This suggests that higher LAI early in the season may contribute to higher yields.

Developmental changes in top FW and plant heights were similar in several respects to LAI alterations. During the early part of the season, heights and top FW of each cultivar grown in Mesa were greater than those in Marana (compare Figs. 6, 7, and 8). After approximately 80 days, however, top FW of cultivars grown in Marana became similar to those grown in Mesa. In the case of 'Norgold Russet' and 'Denali', top FW began to plateau at about 90 days; in contrast, top FW of 'Kennebec' continued to rise.

Comparisons of heights showed that 'Kennebec' plants grown in Mesa were taller than those grown in Marana (Fig. 6) and plants continued to grow until final harvest. Plant heights of 'Norgold Russet' (Fig. 7) and 'Denali' (Fig. 8) plants grown in Mesa were also greater than those grown in Marana during early and mid development. However, heights during late season were similar in the two regions.

Although no attempts were made to correlate LAI or plant height to growth, it seems possible that higher yields of 'Kennebec' may in part be due to higher photosynthetic capacity and vigor. Similarly, yield differences between Mesa and Marana may also be linked to differences in photosynthetic capacity.

Gandar and Tanner (1976a, 1976b) examined the relationship of tuber growth to leaf water potentials in 'Russet Burbank' potatoes and found that growth of tubers was adversely affected by even mild water stress. In their greenhouse study, they noted that well-watered plants had leaf water potentials of -2 bars, and stress affected by withholding water from the soil led to cessation of tuber growth when leaf water potentials decreased to -5 bars. In Arizona, however, tubers grow under conditions of high heat load and evaporative demand. To assess the relationship of leaf water status and temperature to tuber growth, measurements of leaf water potentials and temperatures were made throughout an essentially cloudless day 107 days after planting in Mesa (June 19) and 98 days after planting in Marana (June 29).

At both Mesa (Fig. 9) and Marana (Fig. 10), leaf temperatures during daylight hours generally lay between air and soil temperatures (upper curves). In all cultivars, upper leaves directly exposed to sunlight had higher temperatures than the lowermost leaves closest to the soil and no conspicuous differences were found among cultivars. At both sites also, leaf water potential values during daylight hours tended to be related inversely to leaf temperatures (Figs. 9 and 10, lower curves) and distinct differences among cultivars were also not

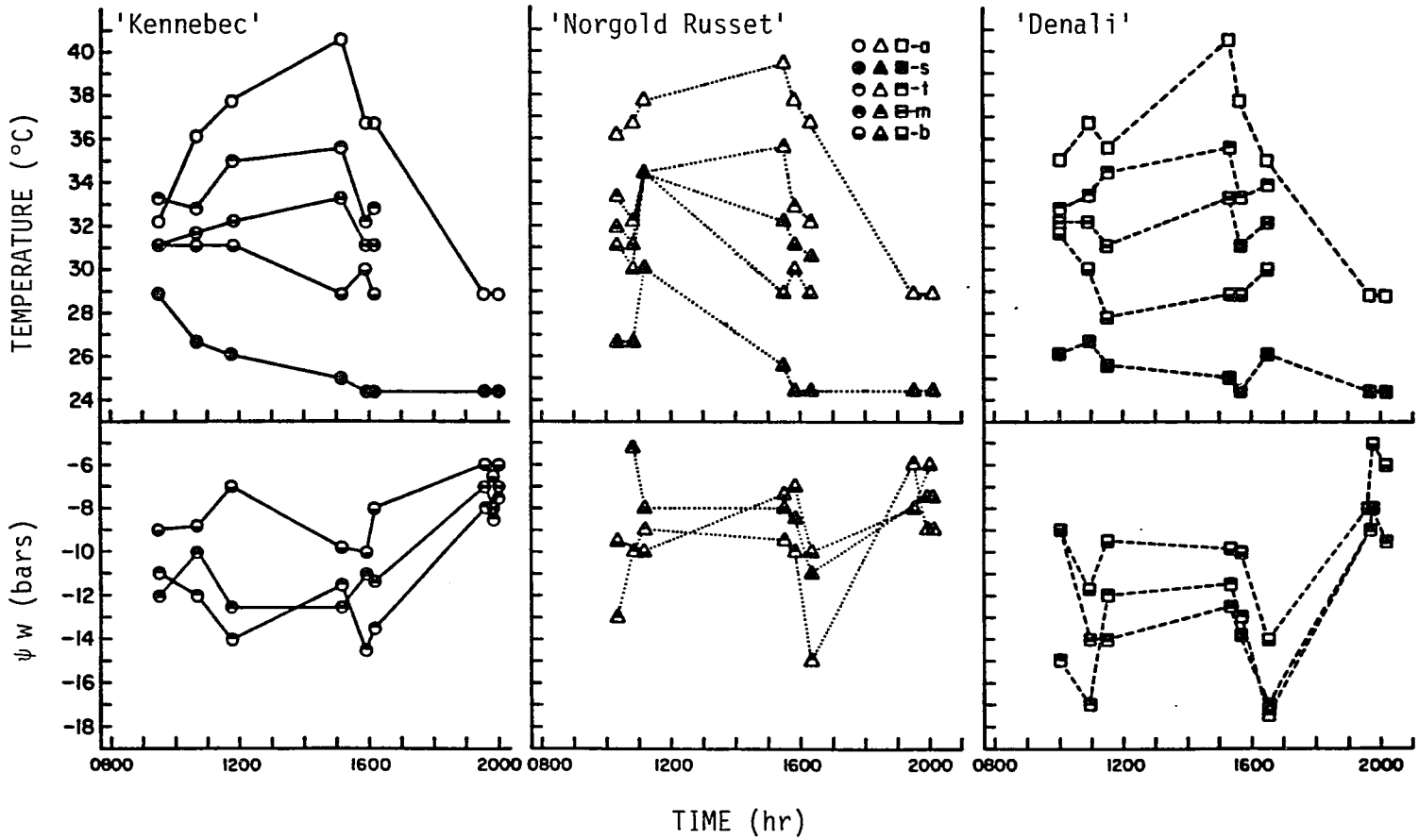



Figure 9. Soil and leaf temperatures and leaf water potential changes occurring during the course of a day at Mesa, 1980. -- a = ambient, s = soil temperatures, t = top leaves, m = middle leaves, and b = basal leaves.

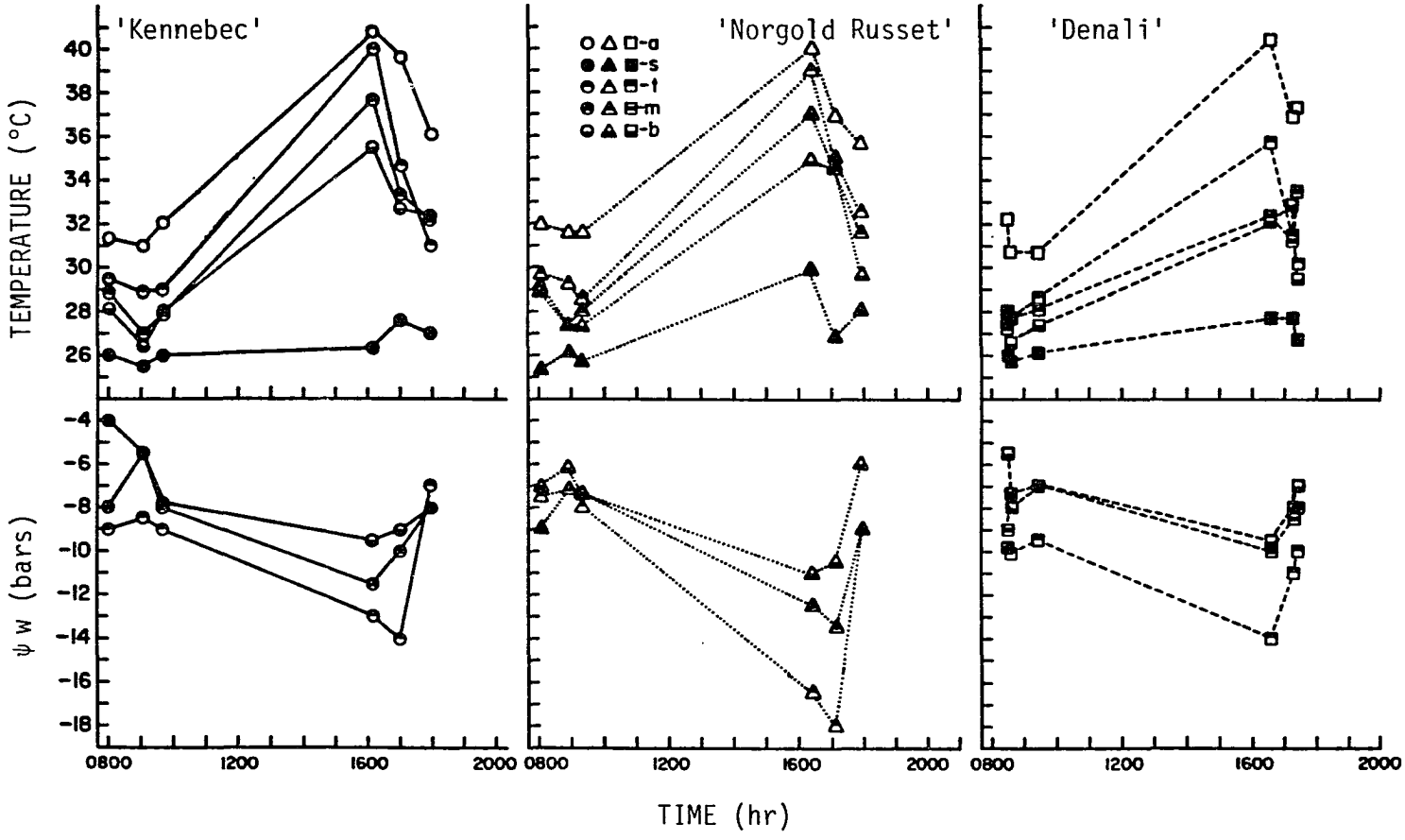



Figure 10. Daily changes of soil and leaf temperatures and leaf water potentials in relation to leaf temperatures of cultivars grown in Marana, 1980. -- a = ambient, s = soil temperatures, t = top leaves, m = middle leaves, and b = basal leaves.

found. In both Mesa (Fig. 9) and Marana (Fig. 10), water potentials were highest at either late night or early morning samplings and were usually between -6 to -8 bars, but by 4:00 p.m. water potential values dropped to -15 bars or lower in the upper leaves and to -10 bars in the lower leaves. Because the soils were well watered, the low water potential values seen in potato leaves were probably a reflection of transpirational water loss exceeding absorption rates.

The water potential values seen even in the early morning hours were generally lower than the -5 bars that Gandar and Tanner (1976a, 1976b) stated was the threshold level below which tuber growth did not occur. This suggests that potatoes grown in Arizona have lower water potential threshold levels or they have adaptive mechanisms that permit tuber growth even though leaf water potential values are low (Van Loon, 1981).

### Changes in Chemical Composition of Tubers during Development (Mesa and Marana, 1980)

In Mesa, all 3 cultivars had about 2 to 3 tubers per plant on April 26 (52 days after planting) and a stabilized average of about 6 tubers per plant after May 6 (63 days after planting). Although the data are not shown, tuber numbers varied from 4 to 9 per plant at each harvest and differences were not seen among cultivars. Similar results were obtained in Marana, which suggested that rapid tuber growth after 85 days (May 28) was due to increase in tuber size rather than tuber numbers.

Sowokinos (1971, 1973) reported that both good ('Kennebec' and 'Norchip') and poor ('Chieftan' and 'Red Pontiac') chipping cultivars grown in Red River Valley, Minnesota increased their sucrose and starch content until tubers reached optimal size (about 90 days after planting). Sucrose levels subsequently fell, but starch values remained constant after 90 days. He also showed that reducing sugars were generally maintained at low levels throughout tuber growth. Additionally, Iritani (1981) showed that water stress during growth has detrimental effects on dry matter and starch content of tubers. He also stated that reduced starch accumulation and increased soluble sugars (especially sucrose) adversely affects quality of tubers during storage. Because potatoes are grown in Arizona under conditions of high heat load and apparent water stress, carbohydrate changes occurring during tuber growth were also studied.

At both Mesa and Marana, TSS levels declined with development of the tuber, but levels in the cultivar 'Norgold Russet' were consistently higher than in 'Kennebec' or 'Denali' (Fig. 11). When components of TSS were examined, it was found that glucose levels in all cultivars declined more rapidly than sucrose at both sites, and by final harvest sucrose was the only remaining soluble sugar (Table 9). At final harvest, also, sucrose levels of 'Norgold Russet' were about 3%, whereas levels in 'Denali' and 'Kennebec' were 1%.

In Mesa, starch contents of tubers from all cultivars initally increased rapidly from the first sampling (Fig. 12), but percentages tended to level off during the time of rapid tuber growth. In Marana,

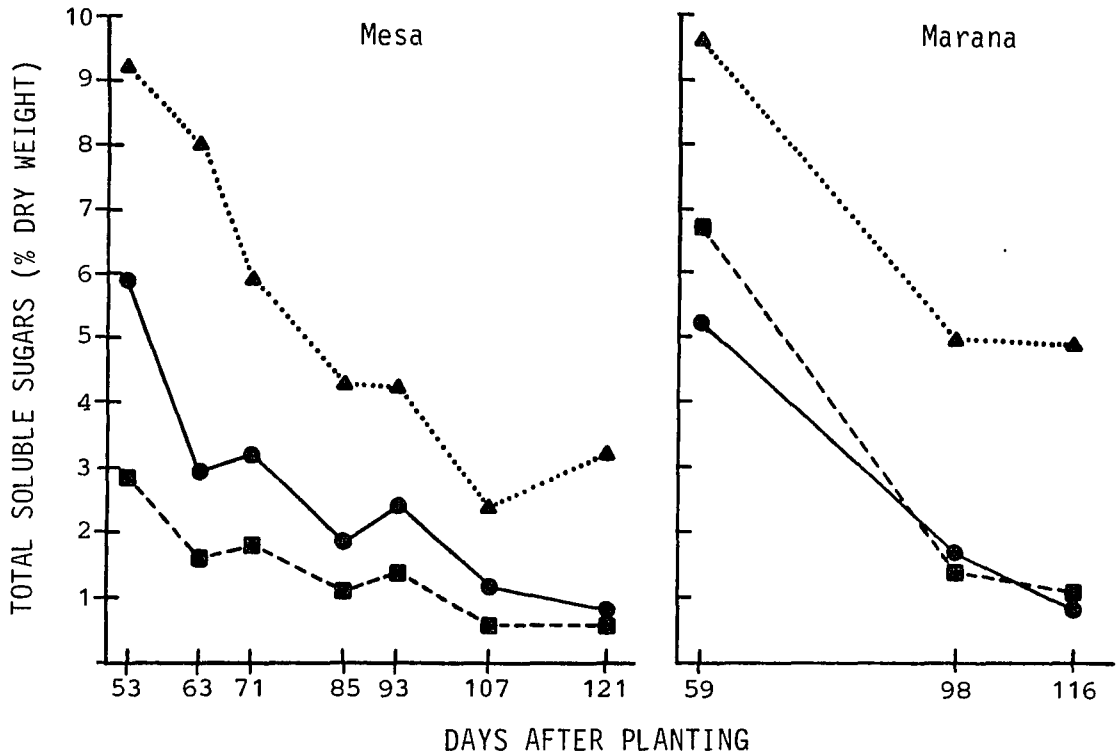



Figure 11. Changes in total soluble sugars of tubers over time in 1980. -- ● = 'Kennebec', ▲ = 'Norgold Russet', and ■ = 'Denali'.

Table 9. Sucrose and glucose content of tubers during development for the potato cultivars 'Kennebec', 'Norgold Russet', and 'Denali'. -- Values represent mean ± SD percent dry weight for 3 determinations.

| Days after Planting | 'Kennebec' | | 'Norgold' | | 'Denali' | |
|---|---|---|---|---|---|---|
| | Sucrose (%) | Glucose (%)[a] | Sucrose (%) | Glucose (%)[a] | Sucrose (%) | Glucose (%)[a] |
| | | | Mesa, 1980 | | | |
| 52 | 2.04 ± 0.22 | 3.97 ± 2.10 | 4.33 ± 2.49 | 4.71 ± 2.36 | 1.96 ± 0.19 | 1.27 ± 0.63 |
| 63 | 2.60 ± 0.18 | 0.76 ± 0.59 | 6.68 ± 0.37 | 1.76 ± 0.42 | 1.53 ± 0.55 | 0.15 ± 0.26 |
| 71 | 2.69 ± 0.61 | 0.35 ± 0.07 | 5.17 ± 0.07 | 0.43 ± 0.08 | 1.64 ± 0.17 | 0.11 ± 0.07 |
| 85 | 1.60 ± 0.35 | ND | 3.66 ± 0.36 | ND | 0.94 ± 0.21 | ND |
| 93 | 2.60 ± 0.74 | ND | 4.16 ± 0.30 | ND | 1.26 ± 0.10 | ND |
| 107 | 1.12 ± 0.25 | ND | 2.29 ± 0.47 | ND | 0.72 ± 0.06 | ND |
| 121 | 0.96 ± 0.23 | ND | 3.02 ± 0.47 | ND | 0.83 ± 0.17 | ND |
| | | | Marana, 1980 | | | |
| 59 | 3.35 ± 0.76 | 1.80 ± 0.34 | 6.85 ± 1.21 | 4.40 ± 1.38 | 4.23 ± 0.59 | 2.68 ± 0.05 |
| 98 | 1.43 ± 0.07 | 0.53 ± 0.02 | 4.19 ± 0.42 | 1.30 ± 0.17 | 1.38 ± 0.36 | 0.40 ± 0.07 |
| 116 | 0.71 ± 0.28 | ND | 2.88 ± 1.40 | 2.0 ± 0.20 | 0.91 ± 0.23 | ND |

a. ND indicates not detectable.

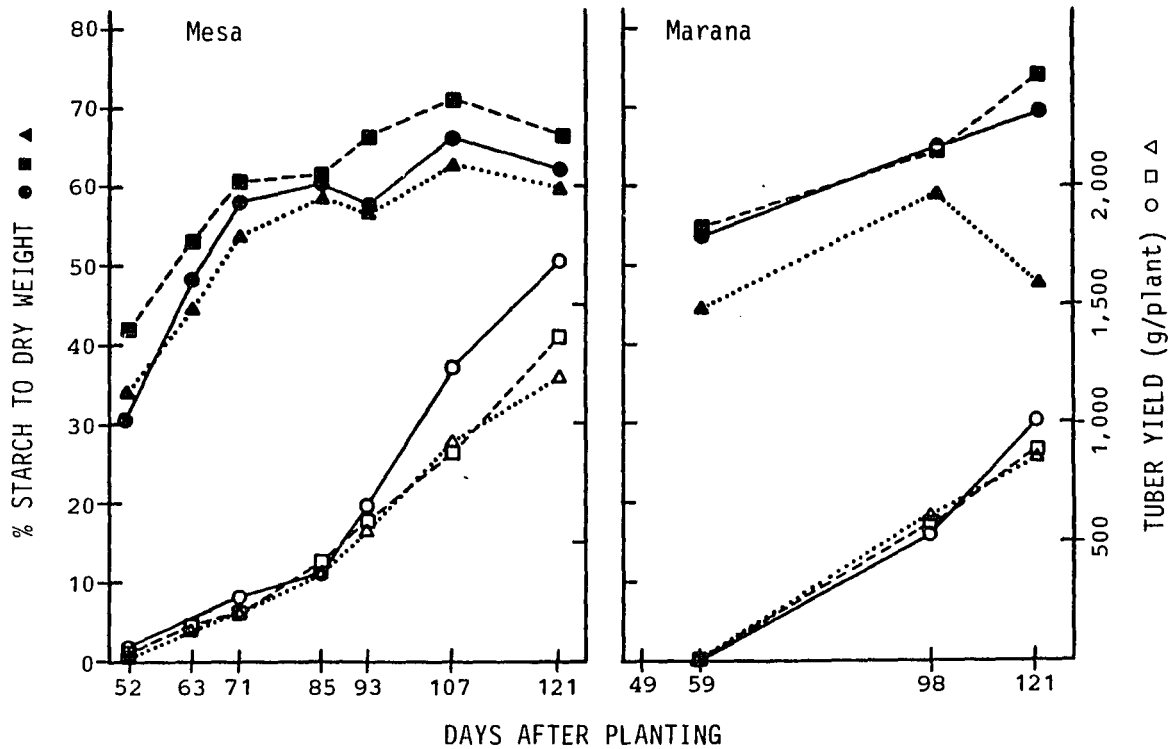



Figure 12. Time course changes in tuber fresh weight and starch percentages during 1980. -- ● = 'Kennebec', ▲ = 'Norgold Russet', ● = 'Denali'.

where sampling periods were fewer, starch percentages showed a linear increase in tubers of 'Denali' and 'Kennebec', but percentages in tubers of 'Norgold Russet' showed a decline at final harvest (Fig. 12).

Average tuber FW of all cultivars grown in Mesa were similar at 93 days after planting, and tuber yields were only slightly larger than those seen in Marana at 98 days after planting (Fig. 12). However, tuber growth in Mesa later in the season was much greater than at Marana, and growth of 'Kennebec' at Mesa was particularly pronounced. Comparisons of tuber DW values (Fig. 13) also showed growth in Mesa during the latter part of the growing season was greater than that seen in Marana. These comparisons suggest that the last month of development is especially critical for tuber yield, and the vigorous late season vegetative growth and high LAI (Fig. 6) values of 'Kennebec' plants grown in Mesa may be one reason why yields for this cultivar were especially high.

## Tuber Yield and Quality at Final Harvest

Growers and processors prefer to have high yielding plants that have tubers with high starch and dry matter and low soluble sugar contents. These attributes can be adversely affected by water stress (Owings, Iritani, and Nagel, 1978). Iritani (1981) concluded that stress results in conversion of starch into soluble sugars which are used to support new shoot growth, a condition resulting in undesirable tubers during storage. Also, Moorby et al. (1975) stated that tubers from plants grown at or under -5 bars for 3 days will be misshapen because of secondary growth. On the other hand, Van Loon (1981) stated

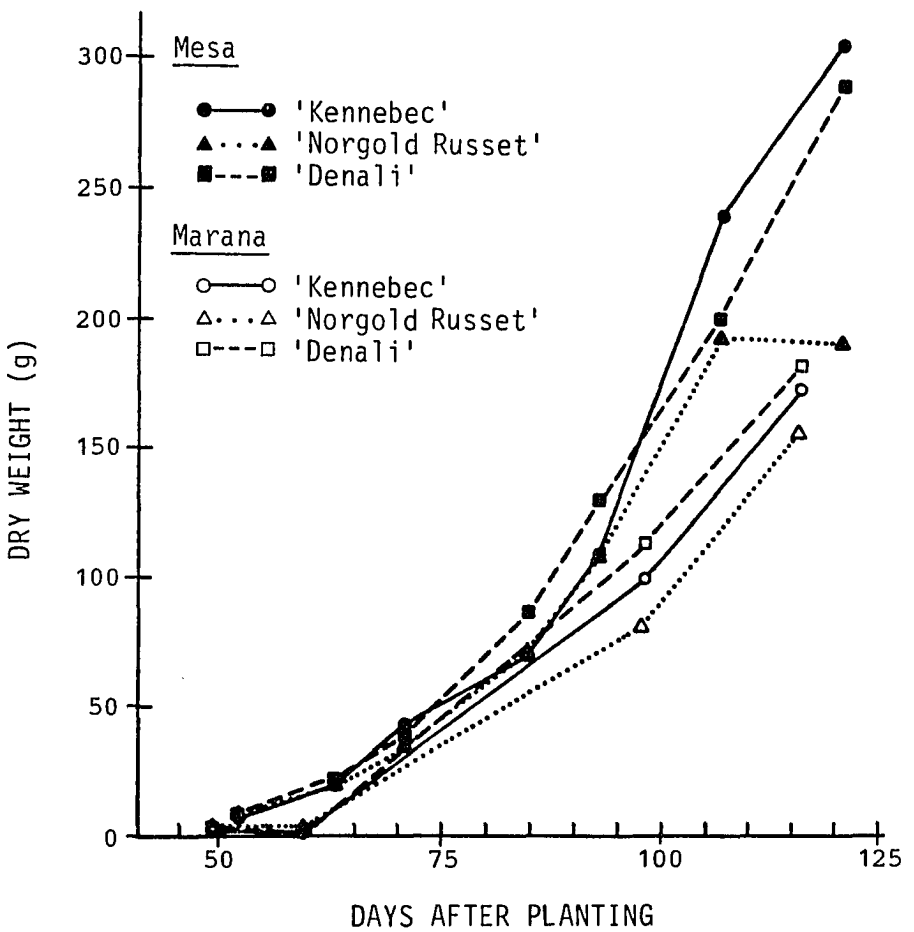



Figure 13. Time course changes in tuber dry weights during 1980

in his review article that under high evaporative demand, plants undergo drought hardening which enables them to increase their net assimilation rate and yield over that of plants grown under wet conditions.

To assess yield and quality attributes of potatoes grown in Arizona under hot, arid conditions, final yield and quality measures were determined for plants grown at Mesa (Table 10) and at Tucson and Marana (Table 11).

Wide variations in yield with surprising little alterations in many other measures were found. Among the cultivars common to all studies ('Kennebec', 'Norgold Russet', and 'Denali'), highest yields were achieved in the 1980 Mesa study (Table 10) and lowest yields were obtained in Tucson during 1979 (Table 11) where waterlogging of the soil occurred. Total and marketable hectare yields of 'Kennebec' and 'Denali' in Mesa during 1980 (Table 10) were above the national average (51 tons total, 33 tons marketable for 'Kennebec'; 37 tons total, 29 tons marketable for 'Denali' (Dearborn, 1979); in contrast, tuber yields in Tucson during 1979 were far below the national average, and marketable yields were essentially nonexistent (Table 11).

To explain why yields in Mesa (Table 10) were substantially greater than those in Marana (Table 11) in 1980, comparisons were made of tuber size classes since all cultivars had approximately 6 tubers per plant. These comparisons showed that each cultivar had approximately the same number of marketable tubers in the 100- to 200-g class, but numbers of tubers that were larger than 200 g were greater in Mesa

Table 10. Yield and tuber quality characteristics at final harvest for potato cultivars grown at the Mesa Experimental Farm, Mesa, Arizona. -- Means in columns followed by the same letter are not significantly different by Duncan's multiple range test, 0.05 level.

| Potato Cultivar | Yield (t/ha) | | % Dry Matter | Specific Gravity | Chipping-Quality Index | Total Soluble Sugars | Starch |
|---|---|---|---|---|---|---|---|
| | Total[a] | Marketable | | | | | |
| | | | 1979 Harvest | | | | |
| 'Kennebec' | NA | 27.27 ab | 18.39 bc | 1.072 b | 2.7 b | 1.54 bc | 67.54 a |
| 'Norgold Russet' | NA | 16.44 c | 16.92 cd | 1.064 c | 5.0 a | 3.79 a | 56.53 c |
| 'Denali' | NA | 21.83 bc | 20.78 a | 1.080 d | 2.0 b | 1.19 c | 66.32 ab |
| 'Red LaSoda' | NA | 24.09 ab | 18.06 d | 1.065 c | 3.0 b | 1.99 b | 65.21 c |
| 'Nebraska S1-3' | NA | 26.77 ab | 16.97 cd | 1.064 e | 3.0 b | 2.38 ab | 56.19 c |
| 'Nebraska 118' | NA | 28.88 a | 20.13 ab | 1.077 a | 2.5 b | 1.39 bc | 60.21 bc |
| | | | 1980 Harvest | | | | |
| 'Kennebec' | 67.56 a | 45.27 | 18.66 a | 1.082 a | 3.5 b | 0.84 b | 62.37 ab |
| 'Norgold Russet' | 47.32 b | 19.40 | 15.55 b | 1.077 b | 6.2 a | 3.19 a | 58.88 b |
| 'Denali' | 54.98 ab | 29.14 | 20.57 a | 1.089 a | 3.0 b | 0.71 b | 66.46 a |

a. NA indicates not available.

Table 11. Yield and tuber quality characteristics at final harvest for potato cultivars grown at the Campbell Avenue Experimental Farm, Tucson, and Marana Experimental Farm, Marana, Arizona. -- Means in columns followed by the same letter are not significantly different by Duncan's multiple range test, 0.05 level.

| Potato Cultivar | Yield (t/ha) | | % Dry Matter | Specific Gravity | Chipping-Quality Index | Total Soluble Sugars | Starch |
|---|---|---|---|---|---|---|---|
| | Total[a] | Marketable | | | | | |
| | | | 1979 Harvest, Tucson | | | | |
| 'Kennebec' | 14.03 a | ND | 19.63 b | 1.078 a | 2.0 b | 2.20 c | 69.58 a |
| 'Norgold Russet' | 7.69 b | ND | 18.06 bc | 1.078 b | 5.0 a | 4.65 a | 54.88 b |
| 'Denali' | 14.78 a | ND | 22.48 a | 1.081 a | 2.0 b | 2.49 c | 67.16 a |
| 'Red LaSoda' | 11.15 a | ND | 18.06 bc | 1.069 c | 3.0 b | 3.14 b | 57.58 b |
| 'Nebraska S1-3' | 14.17 a | ND | 16.14 c | 1.067 c | 3.0 b | 3.70 ab | 54.08 b |
| | | | 1980 Harvest, Marana | | | | |
| 'Kennebec' | 40.70 a | 22.38 | 15.04 ab | 1.063 b | 2.3 b | 0.85 b | 69.52 a |
| 'Norgold Russet | 34.72 a | 10.76 | 12.84 b | 1.062 b | 5.8 a | 4.47 a | 47.77 b |
| 'Denali' | 35.04 a | 11.90 | 18.28 a | 1.072 a | 2.3 b | 1.04 b | 74.39 a |

a. ND indicates not detectable.

than in Marana (Table 12). Numbers of large tubers were particularly high for 'Kennebec' grown in Mesa, where yields were the highest.

Despite the fact there were large variations in yield at different sites and years, the chipping quality of a specific cultivar was not greatly affected. The chipping quality of 'Kennebec' and 'Denali' was always higher than those of 'Norgold Russet' (Tables 10 and 11). Similarly, starch levels of 'Kennebec' and 'Denali' were consistently greater than those of 'Norgold Russet'. Dry matter content and SpG of 'Denali' and 'Kennebec' were also greater than those of 'Norgold Russet', but these values were lower than those of the national average (Dearborn, 1979).

## Changes in Carbohydrate Composition of Tubers during Storage

High temperatures and water stress during development are known to affect the composition of tubers, and these changes are considered to affect potato storage properties. Iritani and Weller (1977) and Iritani (1981) reported that water stress during tuber development reduced starch accumulation and increased reducing sugar content of tubers. The increase in reducing sugar content in tubers affected storability of tubers adversely.

To check storage effects on potatoes grown in Arizona, tubers obtained from the 1979 harvest of Drs. O'Keeffe and Bessey were sampled for various carbohydrate components (1) before and (2) after cold storage for about 1 year at 9°C and 50% relative humidity, and also (3) 12 days after tubers were removed from storage and held at room temperature

Table 12. Comparison of marketable tubers to total tuber yields of potato cultivars grown at the Mesa and Marana Experimental Farms, Arizona

| Potato Cultivar | Mean Fresh Weight of Tubers (g) | Mean Number of Tubers | Ratio Marketable to Total Tubers | Marketable Tubers by Weight Classes: 100-200 g Fraction of Total | 100-200 g Mean Fresh Weight (g) | > 200 g Fraction of Total | > 200 g Mean Fresh Weight (g) |
|---|---|---|---|---|---|---|---|
| | | | Mesa, 1980 | | | | |
| 'Kennebec' | 300 ± 33 | 8 + 2 | .67 ± .16 | .24 | 134 ± 12 | .44 | 386 ± 36 |
| 'Norgold Russet' | 209 ± 4 | 12 ± 2 | .41 ± .06 | .20 | 145 ± 6 | .22 | 267 ± 30 |
| 'Denali' | 219 ± 39 | 11 ± 3 | .53 ± .18 | .23 | 138 ± 9 | .26 | 273 ± 30 |
| | | | Marana, 1980 | | | | |
| 'Kennebec' | 211 ± 23 | 8 ± 2 | .55 ± .12 | .29 | 135 ± 11 | .23 | 313 ± 61 |
| 'Norgold Russet' | 185 ± 22 | 10 ± 3 | .31 ± .19 | .18 | 147 ± 9 | .13 | 245 ± 8 |
| 'Denali' | 215 ± 17 | 8 ± 2 | .43 ± .11 | .20 | 151 ± 9 | .16 | 176 ± 14 |

(26°C). Prior to storage, tubers were cured by holding at room temperature at about 60% relative humidity for 3 days.

One year's storage led to a substantial decrease in starch and a pronounced increase in soluble sugars in all cultivars (compare Table 13a with Table 10). Cultivars that had the lowest soluble sugars and highest starch levels at harvest ('Kennebec', 'Denali', and 'Neb. 118') also maintained the lowest sugars and highest starches after storage. Although the component sugars were not analyzed in the tubers collected immediately after harvest, comparisons made with data from the 1980 harvest (Table 9) indicated glucose levels were unchanged by storage, but sucrose and fructose were selectively increased. The increase in dry matter content obtained after 1 year's storage is likely due to water loss.

When tubers were removed from storage and held at 26°C for 12 days, considerable rotting occurred and about half of the tubers were discarded. The surviving tubers, except 'Red LaSoda', had wrinkled skins and newly emergent sprouts, and almost all tubers had "hollow heart"; therefore, sampling from the pith was omitted. All cultivars except 'Red LaSoda' lost a substantial amount of water and thereby increased in dry matter percentages. The tubers also had reduced TSS, but this reduction may reflect the fact that the pith was not sampled (Table 13b).

## Abnormalities Observed in the Field

Pew et al. (1976) reported that high temperature during tuber development is responsible for tuber cracking and "hollow heart" or "black heart" formation. Additionally, high temperature induced water

Table 13. Sugar and starch content of potato tubers after cold storage for 1 year. -- Means in columns followed by the same letter are not significantly different by Duncan's multiple range test, 0.05 level.

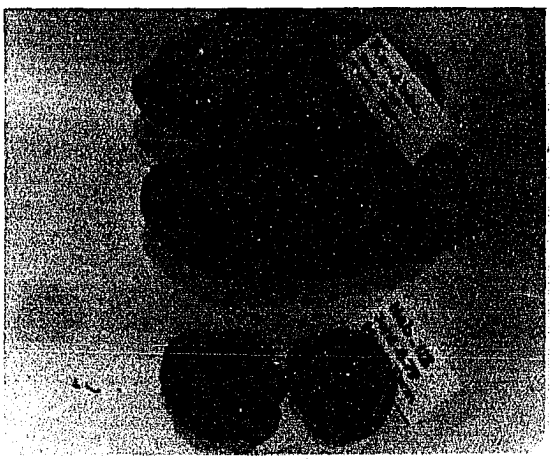
| Potato Cultivar | Total Soluble Sugars | Sucrose | Glucose | Fructose | Starch | % DM |
|---|---|---|---|---|---|---|
| a. Analysis of tubers immediately after storage | | | | | | |
| 'Kennebec' | 18.09 b | 7.33 c | 0.75 bc | 10.01 b | 54.77 c | 28 |
| 'Norgold Russet' | 26.52 a | 16.07 a | 0.84 b | 9.61 bc | 42.23 d | 27 |
| 'Denali' | 12.84 d | 7.71 c | 0.38 d | 4.75 e | 59.38 b | 32 |
| 'Red LaSoda' | 20.84 c | 4.55 e | 1.08 a | 15.21 a | 43.56 d | 26 |
| 'Nebraska S1-3' | 22.68 c | 13.91 b | 1.17 a | 7.60 d | 34.02 e | 24 |
| 'Nebraska 118' | 16.76 b | 6.66 d | 0.66 c | 9.44 c | 74.00 a | 29 |
| b. Analysis of tubers 12 days afer removal from storage | | | | | | |
| 'Kennebec' | 13.77 b | 8.58 b | 0.54 b | 4.65 c | 50.33 d | 50 |
| 'Norgold Russet' | 21.67 a | 12.19 a | 0.80 a | 8.68 a | 50.57 f | 56 |
| 'Denali' | 9.82 c | 5.02 bc | 0.14 c | 1.66 e | 65.68 b | 49 |
| 'Red LaSoda' | 14.42 b | 7.14 c | 0.66 b | 6.62 b | 60.46 c | 31 |
| 'Nebraska S1-3' | 20.80 a | 11.74 a | 0.82 a | 8.24 a | 47.94 e | 56 |
| 'Nebraska 118' | 8.25 d | 4.99 d | 0.20 c | 3.06 d | 68.50 a | 55 |

stress during tuber development may lower SpG and cause the formation of misshapen tubers due to secondary growth (Moorby and Milthrope, 1975; Moorby et al., 1975). Moorby and Milthorpe also stated that "glassy tubers" and rotting may form because of reduced starch and increased glucose, and Smith (1968) reported that high sugar levels of potatoes grown under water stress may also reduce chipping quality.

Examinations of tubers produced in Arizona, however, showed most of the above-suggested relationships did not hold. Specific gravities and % DM were slightly lower than those found in the northern regions of the United States, but there was no conspicuous difference in storage properties or chipping characteristics. Also, no "hollow heart" was found in the 1980 harvests, but "hollow heart" and "glassy tubers' (Fig. 14) were found in the late planting study conducted at Mesa during 1979. The yield reductions seen on and after June 5 (Table 4) were due to this physiological disease. This is likely due to high temperatures during tuber development because the phenomenon was not observed in the O'Keeffe and Bessey field, which was planted earlier (February 9) and harvested on June 8.

In addition to "hollow heart" and "black heart" associated with tubers, dry segments up to about 10 cm in length were frequently seen in the middle-to-upper parts of the primary stems of late planted 'Norgold Russet' plants. The plants, however, showed no conspicuous wilting of the upper parts, but there appeared to be a greater association of "glassy tubers" with these plants. The cause for this disease is still unknown.

a.



b.

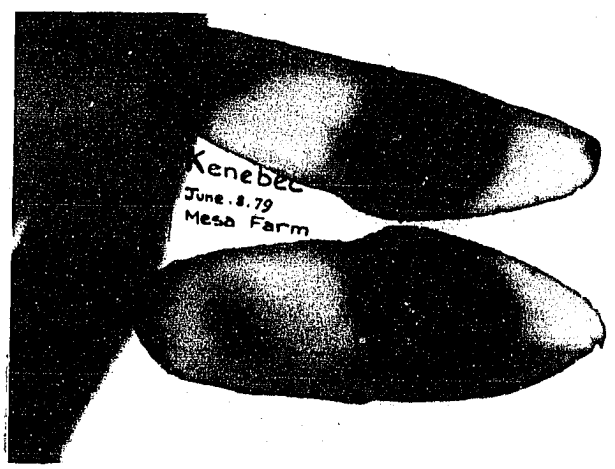



Figure 14. Examples of "hollow heart" or "black heart". -- a. Cross-sectional view of 'Nebraska 118' desiccated "hollow heart" appearance; longitudinal view of 'Kennebec' showing fleshy "hollow heart". b. Detailed view of fleshy "hollow heart". Tubers were found on June 8, 1979 at the Mesa farm.

In Tucson during 1979, the impermeable soil layer virtually eliminated the production of marketable size tubers and the plants were obviously stunted (compare Table 3 with Tables 10 and 11). Additionally, tubers of all cultivars except 'Norgold Russet' were affected by common scap (Streptomyces scabies, Fig. 15a) identified by Dr. Michael Stangellini, Department of Plant Pathology, University of Arizona. Interestingly, this appearance was unusual because the disease is normally present in dry soils and flood irrigation is commonly used as a preventive measure.

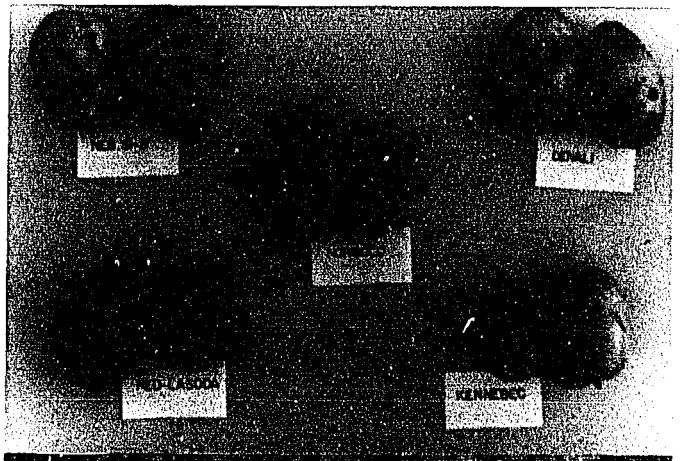
Although 'Norgold Russet' is resistant to common scap, it showed aerial tubers that had internodal thickening similar to that seen with psyllid injury (Fig. 15b). These problems associated with the waterlogged Tucson soil did not affect the chemical composition (compare Table 11 with Table 10) and chip quality of tubers (Fig. 15c).

Psyllids, Paratrioza cockerelli (Sulc), invaded the field after emergence of potato cultivars in 1980 at Mesa. After injury was noticed, insecticide methomyl was used to treat the plants but some sections showed recovery whereas others were permanently injured. Because this presented an opportunity of understanding the physiological effects of psyllids' feeding on potato plants and the results of this study are presented in the following section.

Psyllid Injury in Mesa, 1980

The potato psyllid is a major insect pest of potatoes grown in Arizona (Gerhardt, 1966). In addition to feeding by adult insects, nymphs produce phytotoxins that cause the disease "psyllid yellows".

a.



b.

c.

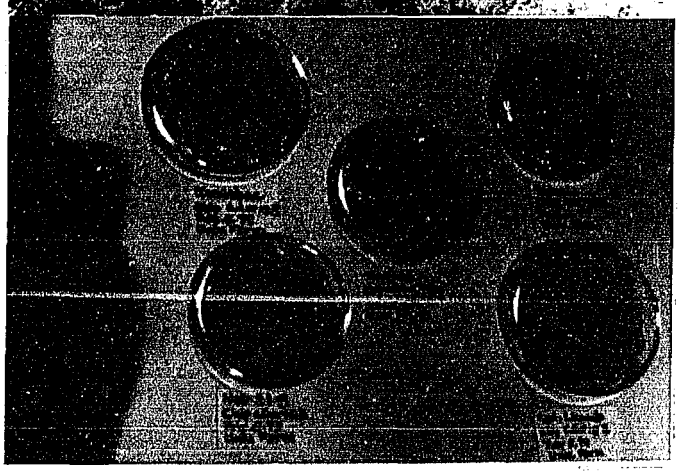


Figure 15. Symptoms of common scap infected plant and tubers, and tuber chipping quality. -- a. Exterior appearance of tubers from different cultivars showing only 'Norgold Russet' tubers were not affected; b. 'Norgold Russet' plants showing formation of aerial tubers, internodal thickening, and leaf formation on the bud end of aerial tubers; c. Shows chipping quality of the cultivars was not affected by common scap infection.

The degree of yellowing is related to the nymph population (Stables, 1968).

In 1980, psyllids invaded a field designed to test relationships between physical and chemical maturity of plants (Sowokinos, 1971, 1973) and yield of potatoes. Three to four weeks after emergence, signs of psyllid injury were noticed throughout the field despite the earlier application of phorate followed with a methomyl spraying 2 weeks later. After spraying to control psyllids, top growth of more than half of the field appeared to regreen and assume normal growth. These plants were classifiied as being "recovered" even though misshapen tubers were found, especially in the cultivar 'Kennebec'. Potatoes growing in areas of the field that were severely affected initially remained a pale green and were stunted during the entire growth cycle. Aerial tubers frequently formed on potatoes from these areas. These plants were considered to be "permanently injured". The specific changes in growth and carbohydrate characteristics of recovered and permanently injured plants were observed and the results of these comparisons are given in this study.

Tuber yields from all plants injured permanently by psyllids were reduced drastically (Fig. 16), but plants that recovered had yields approaching those normally obtained in Arizona. Final yield from recovered 'Kennebec' plants was higher than that of 'Denali' or 'Norgold Russet'.

To determine how psyllid injury reduced yields, measurements were made of various yield and physiological components of permanently

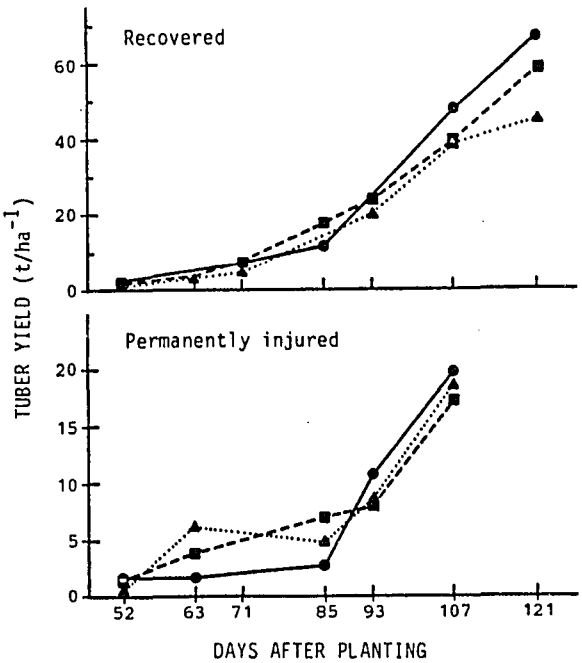
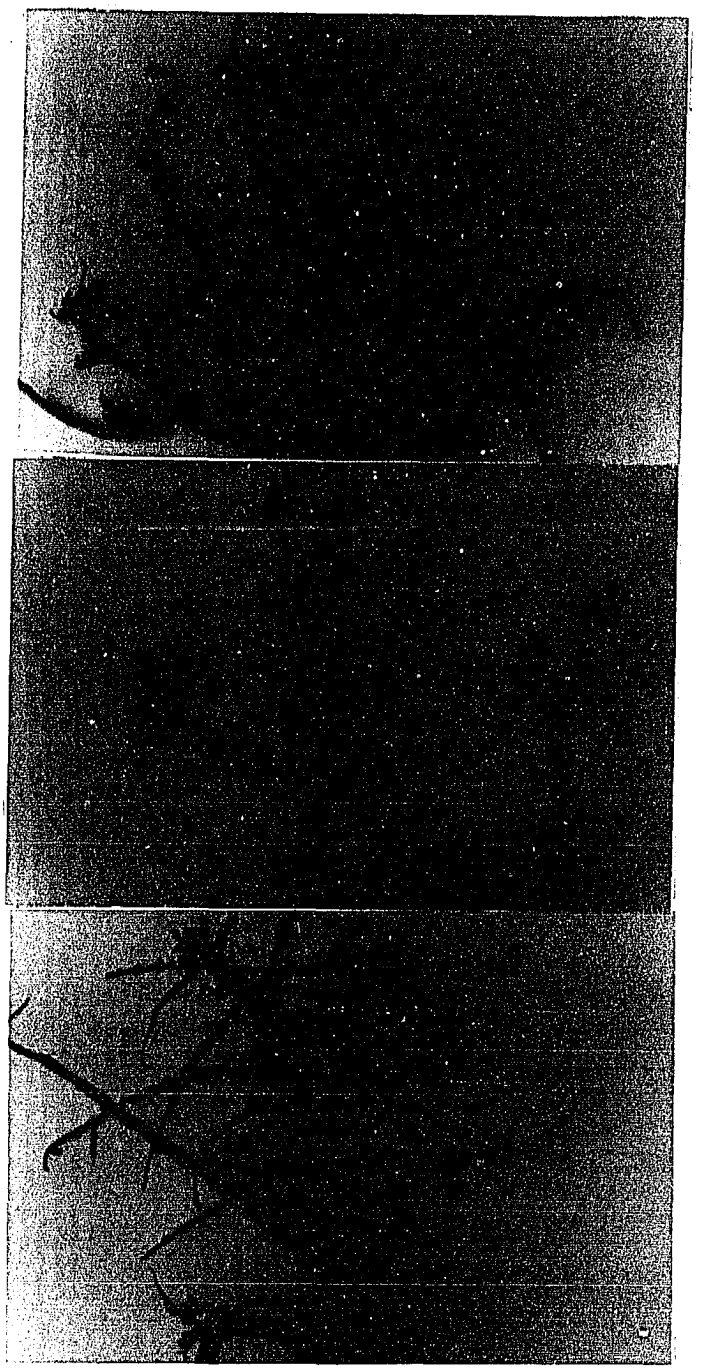



Figure 16. Tuber yields of recovered and permanently injured plants. -- The cultivars are: ● = 'Kennebec', ■ = 'Denali', and ▲ = 'Norgold Russet'.

injured and recovered plants. Plants permanently injured by psyllids were chlorotic from initial injury through senescence. Morphological changes such as aerial tuber formation, rosetted leaves around the bud end of the aerial tubers, and internodal stem thickening were observed. Internodal thickening and aerial tuber formation in 'Norgold Russet' were more frequent than in 'Kennebec' or 'Denali' (Fig. 17c). In addition to these, 'Denali' had new shoot formation on old flower stems (Fig. 17b). Internodal thickening was less in 'Denali' than in either 'Kennebec' or 'Norgold Russet' (Fig. 17b). Leaves dried early (Fig. 18) and lost considerable leaf area (Fig. 19). Because of this early senescence,



a. 'Kennebec'

b. 'Denali'

c. 'Norgold Russet'

Figure 17. Permanently injured 'Kennebec', 'Denali', and 'Norgold Russet' plants. -- a and c show aerial tuber formation, internodal thickening and leaf formation on the bud end of aerial tubers of 'Kennebec' and 'Norgold Russet', respectively. b shows aerial tubers and new shoot formation on old floral stalks in 'Denali'.

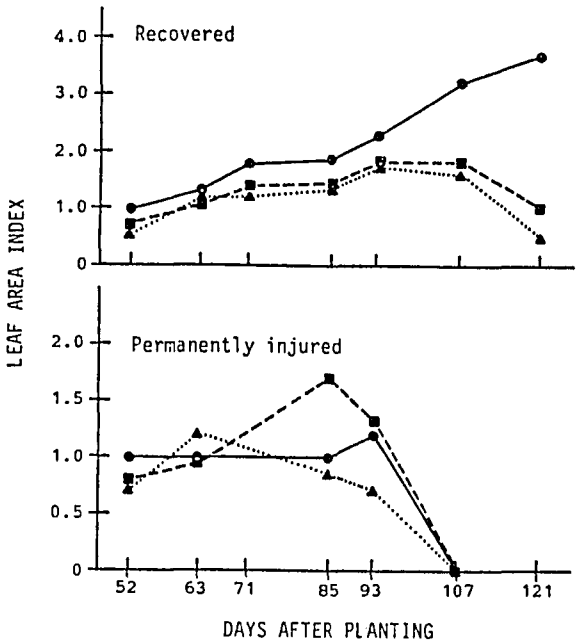



Figure 18. Leaf area index values of recovered and permanently injured plants. -- The cultivars are" ● = 'Kennebec', ■ = 'Denali', and ▲ = 'Norgold Russet'.

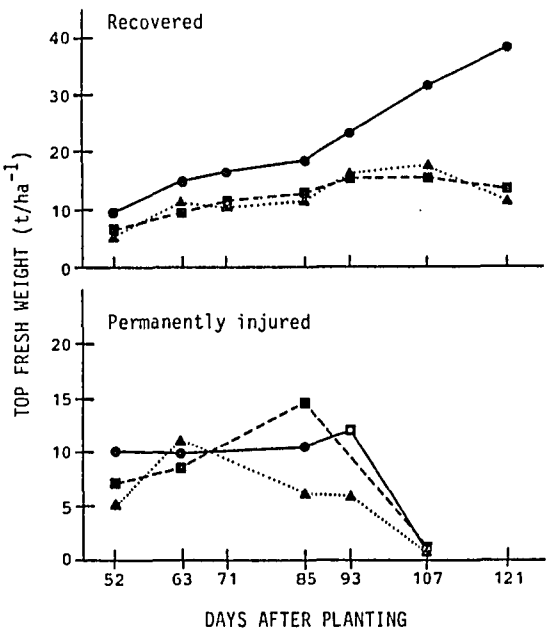



Figure 19. Top fresh weights of recovered and permanently injured plants. -- The cultivars are: ● = 'Kennebec', ■ = 'Norgold Russet', and ▲ = 'Denali'.

tubers of permanently injured plants were ready to harvest 2 weeks earlier than those of plants which recovered (Figs. 18 and 19).

Among the recovered plants, 'Kennebec' showed unique traits that contributed to the relatively higher yields. These plants continued to grow (Fig. 20) and increase in FW (Fig. 20) and leaf area (Fig. 18) until tubers were harvested. With 'Denali' and 'Norgold Russet', these growth characteristics increased slowly or actually declined late in the season. At the time of harvest, leaf and stem FW of the recovered 'Kennebec' plants were clearly greater than those of the other cultivars (Table 14). Additionally, plants that recovered had higher leaf and stem FW than those of the permanently injured plants of the same cultivar.

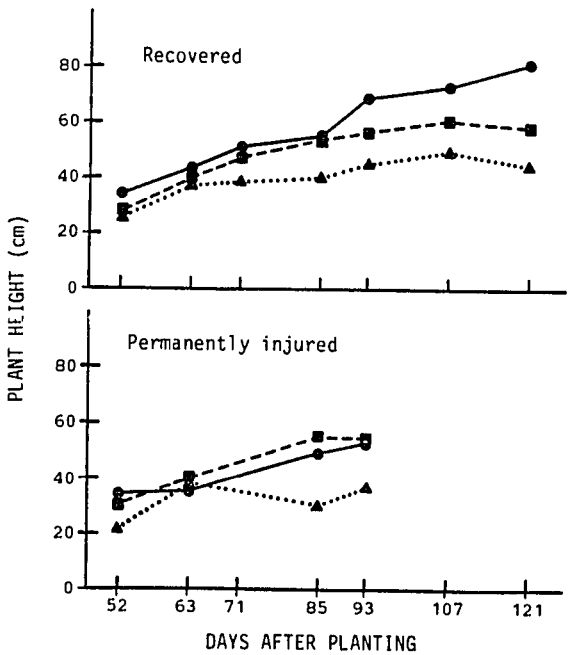



Figure 20. Plant height changes of recovered and permanently injured plants.-- The cultivars are: ● = 'Kennebec', ■ = 'Denali', and ▲ = 'Norgold Russet'.

Table 14. Yield characteristics of 'Kennebec', 'Denali', and 'Norgold Russet' plants. -- Mean of 7 plants.

| Potato Cultivar | Leaf Fresh Weight/Plant (g) | Stem Fresh Weight/Plant (g) | Tuber Yield % of Total Mass |
|---|---|---|---|
| 'Kennebec' Recovered | 331.0 | 367.0 | 60.0 |
| 'Kennebec' Injured | 145.0 | 167.0 | 62.0 |
| 'Denali' Recovered | 202.0 | 156.0 | 73.0 |
| 'Denali' Injured | 164.0 | 121.0 | 50.0 |
| 'Norgold Russet' Recovered | 201.0 | 168.0 | 66.0 |
| 'Norgold Russet' Injured | 85.0 | 65.0 | 63.0 |

No clear psyllid-related pattern was evident in the ratio of tuber weight to leaf weight (Table 14). Tubers represented about 2/3 of the total plant weight in both permanently injured and recovered 'Kennebec' and 'Norgold Russet' plants, and 3/4 of the total weight in recovered and 1/2 the weight of the permanently injured "Denali' plants.

Reduced tuber yield from permanently injured plants reflected lower assimilate availability. Indeed, when tuber yields per plant are studied it is seen that differences in yield between permanently injured and recovered plants are evident from 85 days after planting (Fig. 21). After this time, permanently injured plants increase tuber weights only slightly, whereas major growth of recovered plants occurs in the 85- to 121-day period.

Starch percentage in permanently injured plants showed a pattern similar to those of the recovered plants (Fig. 21). Analysis of soluble sugars showed that 'Norgold Russet' plants had higher TSS levels than those of either 'Denali' of 'Kennebec' in both injured and recovered plants (Fig. 22)

It is presently unclear how psyllid yellows can affect potato plants biochemically to alter growth and yield and why selective recovery of plants occurred following application of methomyl, a systemic insecticide. However, the occurrence of regions which had plants that were permanently injured and other regions where recovery was nearly complete has provided suggestions about how psyllids can reduce potato yield.

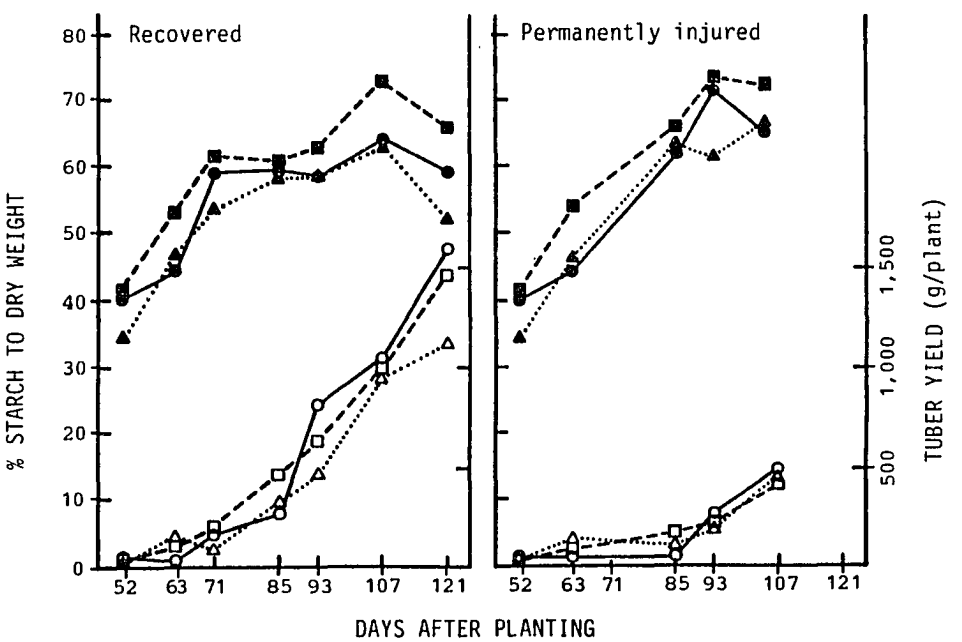



Figure 21. Time course changes in percent starch and tuber yield for recovered and permanently injured plants. -- The cultivars are: ● = 'Kennebec', ■ = 'Denali', and ▲ = 'Norgold Russet'.

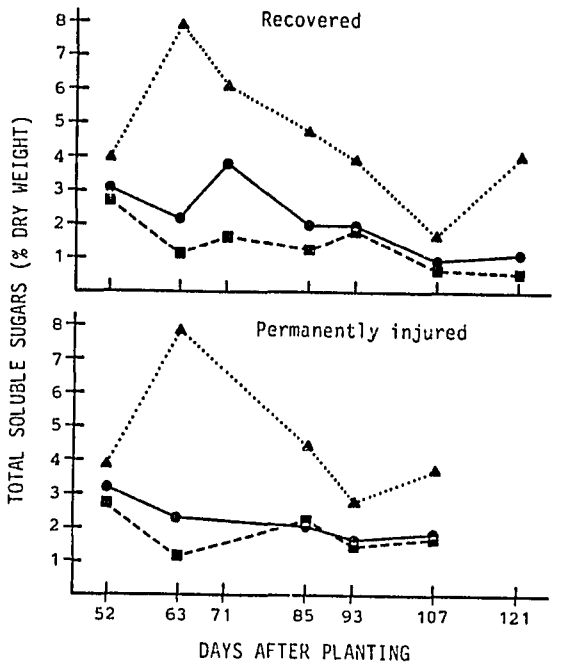



Figure 22. Changes in total soluble sugars of tubers over time of recovered and permanently injured plants. -- The cultivars are: ● = 'Kennebec', ■ = 'Denali', and ▲ = 'Norgold Russet'.

All 3 cultivars appeared to be affected similarly when damage was permanent. Characteristically, stems had much thickened and shortened internodes, leaves were chlorotic, and aerial tubers were frequently formed (Fig. 17). An examination of other diseases with similar symptoms suggests the infection somehow interrupts the vascular continuity to the lower regions of the shoots and thereby causes a reduction in translocation of assimilates to the growing tubers (Peterson and Granovsky, 1950). In terms of tuber development, it appears likely that reduced assimilate supply rather than the presence of any possible toxins found in the upper shoot is the major factor restricting yield. Tubers of

permanently infected plants were small and sometimes misshapen, but the time sequence changes in starch and TSS were not greatly different between the permanently injured and the recovered plants (Figs. 21 and 22). Additionally, the starch to sugar contents generally found for the different cultivars grown in Arizona were maintained. The cultivars 'Kennebec' and 'Denali' have and retain better starch to soluble sugar ratios than 'Norgold Russet' (Figs. 21 and 22).

Although growth regulator studies were not conducted, it is known that regulation of gibberellin and cytokinin levels can influence tuber formation (Chapman, 1958; Gregory, 1956; Hammes and Beyers, 1973; Hammes and Nel, 1975; Kumar and Wareing, 1974; Mauk and Langille, 1978; Mingo-Castel et al., 1976; Palmer and Smith, 1969; Van Staden and Dimalla, 1976). It is presently unknown if changes in growth regulator balance, accumulation of solutes in the upper regions, or other factors are responsible for the chlorosis associated with the disease can also contribute to reductions in tuber yield. Many steps, however, seemed to be involved in the disease phenomena, and one must wonder how toxins per se can affect the dramatic alterations that occur in potato plants. Thus, several phenomena associated with the disease can act in concert to alter productivity.

## Summary and Conclusions

Potatoes are grown commercially in Arizona even though temperatures after mid-April rise to levels that are considered highly inhibitory to tuber production (Bodlaender, 1963). In order to understand the

basis for potato production in Arizona, the relationship of tuber yield and quality to plant physiological properties and growth characteristics were studied in 3 desert regions of Arizona. Seed pieces were planted in early to late March of 1979 and 1980 when average minimum and maximum temperatures were 8°C to 22°C, and tubers were harvested in June and July when minimum and maximum temperatures were 26°C and 42°C. Although the temperatures at planting were satisfactory for growth, the planting dates were approximately 1 month later than sugggested by Pew et al. (1976).

Potato plant and tuber growth development were studied in Tucson in 1979 (planted on March 3) and in Mesa in 1979 (planted on March 17) and 1980 (planted on March 5). At hot temperatures, emergence occurred 3 weeks after planting, and floral buds and tuber initiation were evident 2-3 weeks after emergence. The cultivars 'Kennebec', 'Norgold Russet', and 'Denali' showed flowering on primary stems by 7 weeks and branching and development of axillary stems were evident by 8 weeks. Tuber numbers were maximal (6-10 tubers/plant) and tuber weights were 45 g or over for all cultivars by 9-10 weeks after planting. And by May 21 (11 weeks after planting) maximum temperatures reached to 40°C. Therefore, rapid tuber growth due to cell enlargement occurred at daytime temperature that was above 40°C in Arizona.

Because the 1979 studies in Mesa were harvested before full tuber development occurred and as marketable yields in Tucson during the same year were virutally nonexistent due to the presence of an impermeable soil layer that resulted in water logging, comparisons of development

to tuber yields for 1979 were considered preliminary. Extensive studies conducted in 1980 at Mesa and Marana showed tuber yields at Mesa (planted March 5) were greater than in Marana (planted March 21). In Mesa, tuber yield (tons/ha) of 'Kennebec' (68) were greater than for 'Denali' (55) and 'Norgold Russet' (47); but respective yields in Marana (41, 35, and 35) were not significantly different. High yields of 'Kennebec' when compared to top growth in Mesa high yields were associated with plants that continued to increase in LAI, and plant height late in the growing season when LAI and height of the other cultivars reached a plateau. Leaf area index and plant heights of all cultivars in Mesa were greater than corresponding plant types in Marana, and these and yield differences may be due to higher prevailing temperatures.

In Arizona, plant water status during rapid tuber growth is critical for determining final tuber yields. Temperature and water potential measurements during the period of rapid tuber growth (107 and 98 days after planting in Mesa and Marana, respectively) showed that while daytime temperature reached 42°C, upper and lower canopy leaf temperatures and water potentials were 35°C and 29°C and -15 and -9 bars, respectively. In Marana, air temperatures were also 42°C, and upper and lower leaves had temperatures of 38°C and 32°C and water potentials of -15 and -9 bars, respectively. Because tuber growth probably depends on assimilates from lower (older) leaves, the relatively cool temperatures found there may have allowed yields to be respectable in both Mesa and Marana.

Studies showed that high temperatures during tuber development did not appreciably affect the chemical composition of tubers. Starch percentages of 'Kennebec' (65%) and 'Denali' (73%), recognized as good chipping cultivars, were greater than those of 'Norgold Russet' (59%) in all sites and in both years. After starch levels maximized during tuber development (71 days after planting), percentages of starch did not drop and TSS did not increase during rapid tuber development under high temperature conditions.

Potato tubers are normally processed quickly after field harvest for the purpose of chipping; but potato tubers are also grown for home consumption and storability of tubers for longer duration is desirable. It is known that tuber % DM and TSS content increase upon cold storage (Smith, 1968). Iritani (1981) also reported that qualities of tubers grown under water stress were affected adversely during storage. Storage of Arizona-grown tubers for up to 1 year at 9°C led to about a 25% increase in % DM, but TSS percentages of 'Denali', 'Kennebec', and 'Norgold Russet' increased from 1%, 1%, and 4% to 13%, 18%, and 27%, respectively. At 26°C for 12 days after storage, % DM of tubers was about doubled and TSS sugar values were dropped about 30%. The changes in TSS content of tubers during and after storage were related to the changes in sucrose and fructose content, and glucose content of tubers was less than 1% for all cultivars. At 26°C for 12 days after storage, except for 'Red LaSoda' almost 50% of the cultivar tubers became rotten and the remaining tuber buds emerged and sprouted. 'Red LaSoda' tubers during storage and after storage remained fleshy, but almost all 'Red

LaSoda' tubers developed "hollow heart" after storage and buds were just starting emergence.

Waterlogging and common scap infection of tubers in 1979 in Tucson and psyllid injury in 1980 in Mesa were studied for top growth, tuber yield, and quality. Plant top growth was stunted, and leaves and stems of the cultivars were desiccated. Early drying of the tops during rapid tuber growth resulted in early immature harvest and lower total and marketable tuber yields for the cultivars. However, tuber dry matter content, specific gravity, total soluble sugars, sucrose, glucose, starch content, and chipping quality characteristics were not affected by the waterlogging and common scap infection and psyllid injury (Arslan et al., 1985).

CHAPTER 3

# DEVELOPMENT OF SPECTROPHOTOMETRIC METHODS FOR ANALYSIS OF CARBOHYDRATES IN PLANT, POLLEN, AND INSECT TISSUES

## Introduction

The analysis of carbohydrates in plant and insect tissues is usually difficult or time-consuming, and subject to errors. Early workers extracted carbohydrates with water, and because the conditions favored either enzymatic or microbial degradation, variable results were frequently obtained. To avoid these problems, Reifer and Melville (1947) extracted under ice-cold conditions, whereas Thomas, Melin, and Moore (1949) used 70% ethanol as a solvent, and Williams et al. (1949) extracted with boiling 80% alcohol. Additionally, because tissues contain a variety of potentially interfering substances, partial purifications of extracts were normally performed before further analytical attempts were made. For example, Williams et al. (1979) added water to their alcoholic extract and then treated the aqueous solution with excess neutral lead acetate. The excess lead was later removed by precipitating with dibasic sodium phosphate and filtering. The filtrate was next treated with charcoal or successively with basic and acidic ion exchange resins. However, Rebenfeld and Pascu (1953) noted that strongly basic resins can cause aldose-ketose conversions whereas strongly acidic resins can break down labile (Aspinal et al., 1953) fructofuranoside linkages.

After paper chromatograph (PC) was well developed, Partridge (1948) used this procedure to separate sugars, and Stahl (1965) later resolved sugars with thin layer chromatography (TLC). These nondestructive techniques permit the qualitative separation of sugars and also serve as means for preliminary purification prior to later analysis using spectrophotometric methods or gas liquid chromatography (GLC) or high performance liquid chromatography (HPLC). However, they represent extra steps before sugars can be analyzed, and it is desirable to either eliminate the steps or develop methods that can reduce the time needed for initial purification. Additionally, while GLC and HPLC methods are widely used, some sugars present in plant and insect tissues cannot be well resolved, and the quantities of tissues needed for extraction are relatively large. Accordingly, a concerted effort was made to further investigate the potential of spectrophotometric methods for rapidly determining sugars in tissues.

These studies have shown that many sugars in plant tissues can be analyzed rapidly and accurately with minimal prior separation. Additionally, analysis of sugars present in pollen and bee hemolymph can now also be performed following a simple purification step. Studies used to develop the procedures and the limitations of the methodology are presented in this section.

## Review of Literature

### Introduction

Plant carbohydrates exist largely as cellulose and other β and α glucans, starches, and free soluble sugars present largely as sucrose,

fructose, and glucose (Aspinal et al., 1953). Many insects such as honey bees contain complex carbohydrates like glycogen and soluble sugars that are largely fructose, glucose, and trehalose. Additionally, there are a variety of ions, amino acids, proteins, and other natural products that can interfere with sugar analyses. Many of the interfering materials can be separated using suitable extraction procedures, and solvents such as 80% alcohol can be used to isolate starch from soluble sugars. In spectrophotometric procedures, although little is known about specific reaction mechanisms and the nature of the chromophores, analysts have relied on the differences in stability and reactivity of the different sugars to determine the content of monosaccharides and disaccharides in tissues.

Determination of Monosaccharides

In aqueous solutions, monosaccharides form an equilibrium mixture of open-chain and hemiacetal species, and the latter are considered to be the more active forms (Davidson, 1967). Nelson (1944) and Somogyi (1952) utilized the reactivity of the monosaccharides to determine free fructose and glucose of blood. They deproteinized the blood and later oxidized the monosaccharides with alkaline cuprous ions and followed with arsenomolybdic acid reagent. Because disaccharides are unreactive, this reaction is considered specific for monosaccharides. However, Dische (1962) noted the Nelson-Somogyi procedure is sensitive to air and light, and as a result, blanks were required for every sample.

Glucose has been analyzed by converting to gluconic acid using either glucose oxidase or peroxidase. The $H_2O_2$ formed during the reaction acts as an oxygen donor to weakly nucleophilic compounds such as o-toluidine or o-dianisidine to form blue and orange compounds, respectively. However, nonsugar hydrogen donors such as uric acid, ascorbic acid, and bilirubin can cause an overestimation, and hydrogen ion acceptors such as cysteine can result in an underestimation of glucose. Because the color produced in the assays is stable for only 30 min, the sample numbers used for assays were limited (Fales, Russell, and Fain, 1961).

Aldohexoses, but not ketohexoses, can also react with weak aromatic amines (e.g., o-toluidine) in hot glacial acetic acid to form an equilibrium mixture of colored glycosylamines (Goodwin, 1972). The o-toluidine reaction was previously used for determining serum glucose by Feteris (1965), and it has recently been applied to determine glucose content of barley tissues by Riazi, Matsuda, and Arslan (1985). Because galactose and other aldohexoses will also react with o-toluidine, the presence of these sugars will give an overestimate of the glucose content of tissues. Fortunately, galactose levels are normally low in plants, so o-toluidine can usually provide an accurate estimate of glucose. The reagent, however, is toxic, and all reactions should be performed in the hood.

Determination of Total Soluble Sugars, Sucrose, and Starch

Total soluble sugars (TSS) can also be estimated spectrophotometrically. Monosaccharides, disaccharides, and oligosaccharides are labile to various degrees to strong acids, and hexose units will undergo dehydration to form methyl furfural derivatives of glucose or fructose. These derivatives are unstable and decompose to levulinic acid (Migrdichian, 1957), but methyl furfural derived from either aldohexoses or ketohexoses have free carbonyl groups that can complex with aromatic ketones such as anthrone (Dreywood, 1946) or aromatic alcohols such as phenol (Dubois et al., 1951) to form stable chromogenic compounds (Yemm and Willis, 1954). The hemiacetal nature of sugars makes this reaction specific for carbohydrates, and chloride and ascorbic acid are known to enhance the reaction (Fales et al., 1961). Yemm and Willis (1954) also noted that the reactivity of the hexoses was far greater than those of the pentoses, so the former could be analyzed in the presence of pentoses if reaction conditions are appropriate. However, they also noted that plant tissues could have pigment glycosides, which can yield an overestimation of the soluble free sugars. In broad beans, for example, Yemm and Willis found that such glycosides can comprise 14% to 53% of the TSS, and to avoid problems due to these contaminants they first separated sugars using PC before analysis with the anthrone reagent.

Van Handel (1968) utilized the anthrone reaction to selectively determine sucrose content in mixtures of sugars. He used alkaline conditions to enolize and remove hexoses prior to the anthrone reaction, and then he determined the sucrose content. This procedure has been applied

to insect tissues, but some misinterpretation of data has occurred. Aboul-Nassr and Bassal (1971) and Freyha et al. (1974) concluded that tick hemolymph does not contain trehalose (a nonreducing glucose-glucose disaccharide) but rather contains sucrose. Later Bassal (1973) retracted his conclusion, and he and Barker and Lehner (1976) showed the presence of trehalose rather than sucrose in the coxal fluid of ticks. Modified procedures have now been developed for the assay of trehalose in bee hemolymph (Arslan, Standifer, and Don, 1986) and sucrose in plant tissues (Riazi et al., 1985).

Starch determinations can also be made using the anthrone reaction. Starch, present as the insoluble carbohydrate after 80% alcohol extraction of soluble sugars, was first hydrolyzed with perchloric acid (McCready et al., 1950) and then analyzed. However, Greub and Wedin (1969) concluded that direct use of anthrone reagent without enzymatic hydrolysis resulted in the underestimation of starches in legume roots. Arslan et al. (1985) determined the starch content of potato tubers after enzymatic hydrolysis and subsequent reaction of glucose with o-toluidine.

There are other potential reactions that can be used to analyze for the sugar content in plant and insect tissues, but they have generally not been applied.

Migrdichian (1957) referred to old literature which states that sugar acetates can be prepared by reacting acetic anhydride with sugars in the presence of catalysts such as sodium acetate, zinc chloride, or sulfuric acid. Potentially, these acetates can be coupled

with compounds such as carbazole or phenol-containing reagents to yield colored substances.

Waravdekar and Saslaw (1959) reacted 2-thiobartiburic acid (TBA) with malonylaldehyde under alkaline conditions to obtain a colored substance. TBA was also used in the presence of 9M phosphoric acid + 0.1 N sulfuric acid to colorimetrically determine sialic acid in human serum (Warren, 1959). Percheron (1962, 1967) found TBA could also be used to identify ketoses on TLC plates. Potentially, this reaction can also be used to quantitatively determine ketoses in solution.

Many hydrazones of aldehydes and ketones are colored insoluble substances, but it has long been known that colored glyoxal bis (benzoylhydrazone) and pyruvaldehyde bis (benzoylhydrazone) of sugars are soluble. Russel and Lyons (1969) suggested that yields can be maximized if the reaction with para hydroxybenzoic acid hydrazide (p-HBAH) to form the hydrazones are undertaken in 1% potassium hydroxide (KOH) in 95% alcohol. Lever (1972, 1977) suggested that carbohydrates capable of forming osozones can also yield bis (benzoylhydrazone) in dilute alkaline solutions of p-HBAH. He utilized this reaction to determine glucose in blood.

## Materials and Methods

### Reagents

1. Anthrone Reagent

A stock solution of sulfuric acid was prepared according to Yemm and Willis (1954) by adding 1,500 ml concentrated acid to 600 ml water, and for assays, 200 mg anthrone (Matheson, Coleman, and Bell) was

added to 100 ml aliquots. To ensure reproducibility, stirring was extended over a period of 15 min with slow stirring to eliminate the bubbles formed in the reagent. This procedure and resulting absence of bubbles allows immediate reactions of anthrone with sugars without prior storage in the refrigerator, as suggested by Yemm and Willis (1954). This reagent was used for many studies, but since studies showed that color was enhanced by the presence of alcohol, later stock solutions of the sulfuric acid contained 40 ml absolute alcohol for each 2 L.

2. Carbazole Reagent

Ninety milliliters of glacial acetic acid were made to 100 ml with acetic anhydride, and 10 ml concentrated sulfuric acid were cautiously added. This was followed by 250 mg sodium acetate and 100 mg carbazole (Matheson, Coleman, and Bell).

3. Phenol Acetate Reagent

This reagent was prepared identically to that shown above for the carbazole reagent except that 3 to 4 ml water-saturated phenol containing 0.1% 8-hydroxyquinoline was substituted for the carbazole.

4. Thiobarbituric Acid Reagent

A solution of 10 ml absolute ethanol in 30 ml concentrated hydrochloric acid (HCl) was added with shaking to a mixture of 45 ml water and 15 ml absolute ethanol, and 100-200 mg of TBA (Aldrich) was then added. The reagent was prepared immediately before assays and was used within 30 min.

5. p-Hydroxybenzoic Acid Hydrazide Reagent

p-HBAH (Aldrich) (0.76 g) was dissolved in 100 ml of 80% ethanol, which contained 0.5% NaOH or 100 ml 50% ethanol, or distilled water that contained 0.5%, 1%, or 2% NaOH (modifications of Lever, 1972, 1977).

6. o-Toluidine and o-Ethylaniline Reagents

Both reagents were prepared according to Goodwin (1972). Sixty milliliters of o-toluidine (Eastman Kodak) or o-ethylaniline (Eastman Kodak) and 2.0 g thiourea (Sigma) were made to 1 L with glacial acetic acid and were stored in amber bottles in the hood. These reagents are good for several months to a year, but all are toxic.

Sample Preparations

Sample preparations for soluble and insoluble carbohydrate analysis, separation of free soluble sugars and glycosides from ethanol extracts using bonded silica columns, and starch analysis were as follows:

1. Sample Preparation for Soluble and Insoluble Carbohydrate Analysis

Samples for carbohydrate analysis of potato tubers and honeybee (Apis mellifera L.) hemolymph were prepared according to the procedures of Arslan et al. (1985) and Arslan et al. (1986), respectively.

To prepare leaf and pollen samples, 10-30 mg dry tissues were transferred into 13 x 100 mm test tubes and 5 ml 80% ethanol were added, and the tube was shaken (sonicated in the case of pollen) several times over a period of 20 min. Five more milliliters of 80% ethanol were added and the tube was tightly capped, shaken, and held overnight.

The ethanol soluble fraction was used for determining TSS, nonreducing disaccharides (NRD), total keto sugars (TKS), and total reducing sugars (TRS), sucrose, fructose, and glucose, and the insoluble residue was analyzed for starch.

Although analysis of carbohydrates in potato tubers did not require prior removal of glycosides, these steps were required for carbohydrate determinations in pollen, bee hemolymph, and in some plant leaves.

2. Separation of Free Soluble Sugars and Glycosides from Ethanol Extracts using Bonded Silica Columns

After completion of sugar analysis, 5 ml of the remaining alcoholic extract were dried, dissolved in 2 ml distilled water, and passed through a conditioned cyclohexyl (CH) bonded nonpolar silica column cartridge (Analytichem International). The eluate was then passed through a quaternary ammonium bonded ion exchange (SAX) silica column cartridge (Analytichem International). A 2 ml eluate volume was collected and brought to 5 ml with 3 ml anhydrous ethanol to give a 60% alcoholic solution.

3. Sample Preparations for Starch Analysis

Ethanol insoluble residues from pollen, bee feces, and leaves were washed 3 times with 5 ml 80% ethanol and centrifuged at 1,000 rpm in a Sorvall GLC centrifuge with Type M rotor after each wash. The insoluble residue was dried overnight, ground with a glass rod, and 0.6 ml 0.5 N NaOH was added. The mixture was shaken, then 2.4 ml

distilled water was added, heated to 70-80°C for a few seconds and shaken again to disperse the starches. The alkaline suspension was neutralized with 1 ml 0.33 N acetic acid, and 3 ml (1 mg/ml) α-amyloglucosidase enzyme (Sigma) in 0.2 M pH 4.6 acetate buffer was added, and the mixture was incubated in a water bath at 45°C for 3-5 hr (Arslan et al., 1985). Enzyme activity was stopped by heating in a boiling water bath for 2-3 min, and the mixture was cooled, capped, and stored overnight in the refrigerator at 4°C.

Spectrophotometric Procedures

Spectrophotometric procedures were carried out in the following manner:

1. TSS Determination with Anthrone

An aliquot of the ethanol soluble fraction (0.1 ml) was reacted with 3 ml freshly prepared anthrone reagent in a boiling water bath (97°C) for 4 min. Samples were cooled in ice water and read at 625 nm in a Gilford Model 240 spectrophotometer and compared with sucrose standards.

2. TSS Determination with p-HBAH

A 0.1 ml aliquot of the alcoholic extract was first reacted with 20-25 µl 10% methanolic-HCl and shaken, then 5 µl 2% aqueous potassium permanganate ($KMnO_4$) solution was added, shaken again, and test tubes were then heated briefly at 70°C until the brown precipitates settled. After the samples were cooled, 3 ml of p-HBAH reagent was added, shaken, and heated at 75°C for 10 min. Reaction mixtures were

then cooled to room temperature and absorbances were obtained at 420 nm. In addition to plant samples, reactions were conducted with standard glucose, fructose, and sucrose solutions.

3. NRD Determination

Nonreducing disacchrides were analyzed with a 2-step procedure involving an initial elimination of monosaccharides and later analysis of disaccharides. Elimination of monosaccharides was done either by enolization with alakli or by reduction of reducing groups of monosaccharides with borohydrides.

To enolize monosaccharides, 0.1 ml of alcoholic extracts was heated for 25-30 min at 97°C with 0.1 ml KOH (5.4 N for anthrone and TBA reagents, 1.8 N for carbazole and phenol-acetate reagents), then cooled.

The reduction of aldo and keto monosaccharides into corresponding alcohols was done by reacting 0.1 ml of ethanolic extract with 0.1 ml of 0.26 M sodium borohydride ($NaBH_4$) in anhydrous ethanol (or with 0.1 ml 0.19 M potassium borohydride ($KBH_4$) in 75% v/v methanol). The samples were incubated at 70°C for 5 min, cooled, and excess borohydrides were hydrolyzed with 0.1 ml glacial acetic acid.

Disaccharides remaining after elimination of monosaccharides were treated with 3 ml anthrone reagent, heated at 97°C for 4 min, and cooled in ice water and read at 625 nm against sucrose.

4. Sucrose Determination

Samples that were either enolized or reduced were reacted with 3 ml phenol-acetate, carbazole, or TBA reagents. Sample and reagent mixtures were heated at 97°C for 15 min for phenol-acetate and carbazole reagent and 10-12 min for TBA reagent, cooled in ice water, and absorbances were read at 440 nm for phenol-acetate and TBA reagents, and 495 nm for carbazole reagent against sucrose as a standard.

5. TKS Determination

Total keto sugars were determined as outlined for sucrose determination, but samples were not enolized or reduced prior to reaction. Fructose and the fructose moiety of disaccharides were determined against sucrose and fructose as standards.

6. Glucose Determination

An aliquot containing 0.25 to 1.0 ml ethanolic extract was brought to 1 ml with 80% ethanol and mixed with 5 ml either o-toluidine or o-ethylaniline reagent. Samples were then heated at 97°C for 17 min, and absorbances were obtained at 630 nm with Bausch and Lomb Spectronic 20 spectrophotometer. All operations were in the hood and glucose was used as the standard.

7. TRS Determination

Initial estimates of TRS were made by reacting 0.1 ml of extract with 3 ml p-HBAH reagent dissolved in 80% ehtanol containing 2% NaOH. Mixtures were heated at 75°C for 10 min, cooled, then read at 420 nm. However, because some turbidity occurred, other combinations of alcohol

and alkali were used, and later determinations involved reaction of extracts at 97°C with p-HBAH reagent in distilled water containing 0.5% NaOH, and readings were at 395 nm.

8. Starch and Glycogen Determination

After enzymatic hydrolysis of the ethanol insoluble fractions of leaves, pollen, or bee feces (see sample preparation for starch analysis), aliquots of 0.25 to 1 ml were brought to 1 ml with 80% ethanol and reacted with 5 ml o-toluidine or o-ethylaniline to determine starches. Alternatively, 25 to 50 µl aliquots were reacted with p-HBAH reagent to determine starch as described in the TRS determination procedure.

GLC Analysis of Hemolymph

One microliter of 10 µg/ml of myo-inositol was added to 0.2 ml of hemolymph extract. An appropriate trehalose, glucose, and fructose sugar standard mixture containing 1 µl of 10 µg/ml was also prepared and dried under vacuum at 35°C for 18 hr. The sugars then were converted to their volatile trimethylsilyl ethers by reacting with 100 µl TMSI (Pierce) at 65°C for 20 min and injected into a Varian 1700 Aerograph GLC with an FID detector interfaced with Autolab 6300 integrator and a Beckmann 10" recorder. The column was packed with 3% OV-17 on 80/100 GC-Q (1.8 m). Carrier gas was $N_2$ at 15 ml/min with an injector temperature of 225°C, detector temperature of 245°C, and a temperature program of 10°C/min.

TLC and Detection of Spots

Alcoholic extract of plant tissues (10 µl) and 2 µl of each standard sugars were spotted on a TLC plate (Baker Si 250) and each plate was developed twice in the same direction for a given solvent system. Chromatographic chambers were pre-exposed to solvents overnight prior to plate insertion to ensure vapor saturation.

Solvent Systems Used

The solvent systems used were: n-butanol:acetic acid:water (B:A:W; 4:5:1) upper phase (Partridge, 1948) and N,N' dimethyl formamide: n-butanol:water (DMF:B:W; 1:4:1.5) (Wiens and Gilbert, 1967).

Spots were detected with the following spray reagents:

1. p-Anisidine Hydrochloride Reagent

p-Anisidine hydrochloride (3-5%) in n-butanol:ethanol:water (4:1:1) containing a trace of stannous chloride for stabilization and sensitization (Hough and Jones, 1962) or p-anisidine (2.3 g) in concentrated HCl (5 ml), ethanol (30 ml), and n-butanol (60 ml) were sprayed on plates. After plates were air dried, they were heated at 100-120°C for a few min (Wickberg, 1962). Aldohexoses yielded green-brown spots; ketohexoses, yellow spots; aldopentoses, green spots; and uronic acids, red spots.

2. Napthoresorcinol Reagent

The reagent is prepared by mixing together (a) 50 ml of 0.2% solution of napthoresorcinol in anhydrous ethanol, (b) 50 ml of 0.25 N hydrochloric acid, and (c) 5 ml of concentrated orthophosphoric acid.

The mixture was sprayed on the plate, dried, and heated at 105°C for 5 min (Partridge, 1948). Ketoses yielded red spots and aldoses, blue spots.

3. Potassium Permanganate Reagent

A 1% solution of $KMnO_4$ in 2% sodium carbonate and 2% sodium metaperiodate was made up to 100 ml with distilled water. The plates were sprayed, and upon air drying at room temperature carbohydrates developed orange-red spots. Spots were circled as quickly as possible because of rapid color fade (Wiens and Gilbert, 1967).

4. Phenol-Sulfuric Acid Reagent

Phenol (6 ml) and concentrated sulfuric acid (10 ml) were dissolved in 180 ml anhydrous ethanol, and plates were sprayed, dried, and heated 10 to 20 min at 120°C. Carbohydrates produced orange-brown color spots (Touchstone and Dobbins, 1983).

5. 2-Thiobarbituric Acid Reagent

Thiobarbituric acid (0.5%) was dissolved in 98 ml 80% ethanol containing 2.34 ml of 85% orthophosphoric acid. Plates, after spraying and drying, were heated in the oven at 110°C for 5 min. Ketoses and fructose containing oligosaccharides give grass-green color spots, whereas aldoses give blue color (Percheron, 1967).

6. Sulfuric Acid-Thiobarbituric Acid Reagent

Developed plates were sprayed with dilute sulfuric acid ($H_2SO_4$) (5 ml conc. $H_2SO_4$ in 95 ml distilled water), and warm air dried. The

dried plates were then sprayed a second time with TBA reagent, air dried, and spots were revealed at 110°C after 5 min. This reagent is more specific than the phenol-sulfuric acid spray reagent. General carbohydrates and aldoses give a blue color, ketoses give a grass green color, and arabinose gives a purple color (Arslan et al., 1986).

## Results and Discussion

### TSS and NRD Determinations with Anthrone

Anthrone will react with monosaccharides, oligosaccharides, and polysaccharides, but the reactivity varies with the complexity of sugars and also the reaction conditions (Yemm and Willis, 1954). Additionally, this reaction can be combined with others to achieve distinctions between monosaccharides and nonreducing disaccharides (Van Handel, 1968).

In my earlier studies with plant extracts, however, variable data were obtained because of procedural problems involving mixing sequence. The problems were found to be due to use of aqueous extract of sugars, which were difficult to mix properly with the viscous anthrone reagent. This difficulty was resolved by using alcoholic rather than aqueous extracts of the sugars.

In earlier studies (Riazi, Matsuda, and Arslan, 1985), values for sucrose were sometimes equal to or greater than TSS values even though the tissues analyzed were known to contain glucose and fructose as well as sucrose. Since the sucrose determinations involved the use of KOH to enolize and eliminate monosaccharides, studies were conducted to determine if there was an enhancement due to cations.

When KOH was added to sugar solutions and held at room temperatures prior to reaction with anthrone, absorbance values in reactions with sucrose increased (compare Column 2 with Column 1, Table 15). However, color development by fructose was less than in the controls, which was probably due to an apparent selective enolization of the ketose since glucose values were unaffected. Because the anthrone reagent is highly acidic, it appeared likely that anion was responsible for the enhanced sucrose values. This was supported by further studies with potassium ($K^+$) carbonate (data not shown) and rubidium carbonate ($Rb_2CO_3$) (Column 3, Table 15) that showed alkali ion additions effected about a 20% increase in absorbance values of all sugars. Studies with other inorganic compounds showed that 3 mg bismuth acetate in 100 ml anthrone reagent increased absorbance values, but copper sulfate, Benedict's reagent, and rhodium carbonyl chloride had little or no effect on color formation (data not shown). Color development was also stimualted by the use of 40 ml ethanol in 2 L $H_2SO_4$ stock solution, and because this enhancement was no longer affected by the addition of potassium or other ions, alcohol was routinely added to the anthrone reagent in later studies.

As color formation and fade is time dependent, kinetic studies were conducted with several modified anthrone reagents (Table 16). At 97°C maximum color development with alcoholic solutions of sucrose and glucose occurred in 4 min, but development with fructose occurred even earlier (Column 1, Table 16); in contrast, absorbance values with aqueous glucose solutions performed with the Yemm and Willis (1954)

Table 15. Effect of $K^+$ and $Rb^+$ on anthrone reaction as measured by absorbance. -- Sugars in 100 µl of 80% ethanol were reacted with 3 ml anthrone reagent at 97°C for 7 min, or were incubated at 26°C for 25 min with 100 µl 5.4 N KOH (+ KOH), or 100 µl of 2 mg $Rb_2CO_2$ without incubation before reacting with anthrone. Values represent absorbances at 625 nm, and the standard anthrone reaction represents the means of 3 replications.

| Concentration (µg/Rx) | Absorbance Values for Reaction (Rx) Conditions | | |
|---|---|---|---|
| | Standard Rx | + KOH | + $Rb_2CO_3$ |
| | **Sucrose** | | |
| 0 | 0.000 | 0.000 | 0.000 |
| 20 | 0.355 ± 0.02 | 0.429 | 0.419 |
| 40 | 0.679 ± 0.03 | 0.814 | 0.871 |
| 60 | 1.010 ± 0.03 | 1.167 | 1.274 |
| 80 | 1.322 ± 0.04 | 1.545 | 1.698 |
| | **Glucose** | | |
| 0 | 0.000 | 0.000 | 0.000 |
| 20 | 0.308 ± 0.05 | 0.298 | 0.330 |
| 40 | 0.589 ± 0.04 | 0.595 | 0.675 |
| 60 | 0.878 ± 0.03 | --- | 0.956 |
| 80 | 1.140 ± 0.04 | 1.089 | 1.315 |
| | **Fructose** | | |
| 0 | 0.000 | 0.000 | 0.000 |
| 20 | 0.337 ± 0.03 | 0.115 | 0.427 |
| 40 | 0.627 ± 0.02 | 0.278 | 0.812 |
| 60 | 0.914 ± 0.03 | --- | 1.234 |
| 80 | 1.179 ± 0.06 | 0.602 | 1.530 |

Table 16. Reaction kinetics of sugars with modified anthrone reagents. -- Values represent absorbances at 625 nm.

| Concentration (µg/Rx) | Reaction Period (min) | Absorbance Values for Reaction (Rx) Condition[a] | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| | | Sucrose | | |
| 40 | 2 | 0.656 | 0.760 | 0.616 |
| | 4 | 0.784 | 0.909 | 0.663 |
| | 6 | 0.786 | 0.899 | 0.660 |
| | 8 | 0.689 | 0.871 | 0.655 |
| | 10 | 0.637 | 0.850 | 0.606 |
| | 20 | 0.571 | 0.673 | 0.441 |
| | | Fructose | | |
| 40 | 2 | 0.821 | 1.010 | 0.941 |
| | 4 | 0.801 | 0.900 | 0.783 |
| | 6 | 0.717 | 0.820 | 0.674 |
| | 8 | 0.704 | 0.826 | 0.615 |
| | 10 | 0.708 | 0.765 | 0.554 |
| | 20 | 0.558 | 0.610 | 0.381 |
| | | Glucose | | |
| 40 | 2 | 0.450 | 0.419 | 0.147 |
| | 4 | 0.679 | 0.731 | 0.330 |
| | 6 | 0.674 | 0.710 | 0.431 |
| | 8 | 0.643 | 0.694 | 0.491 |
| | 10 | 0.594 | 0.635 | 0.495 |
| | 20 | 0.452 | 0.512 | 0.423 |

a. Reaction conditions:

1. Sugar standards in 100 µl 80% ethanol were reacted with 3 ml anthrone reagent at 97°C for indicated period.

2. $Rb_2CO_3$ (100 µl of 2 mg/ml) was added to sugar standards in alcohol as in Reaction 1, and reacted with 3 ml anthrone reagent containing 1 ml 10 N KOH per 100 ml reagent.

3. Sugar standards were prepared in distilled water and reacted with anthrone reagent according to the method of Yemm and Willis (1954)

procedure peaked in 8-10 min (Column 5) and were lower than with alcoholic solutions. The data of Table 16 show also that significnt color enhancement will also occur with anthrone reagents containing KOH (Column 2). As a result of studies of this type, a 4-min reaction time with alcoholic solutions was routinely used in further studies.

As a supplement to the above study, the reaction mixtures were subsequently held at 26°C or also on ice for periods up to 20 min. As no change in absorbance occurred in the interval (data not shown), it was concluded that final color formed was not affected by holding reaction mixtures at room temperature or on ice for 20 min. This is normally the time required to add reagents to 60 samples.

Results such as those in Tables 15 and 16 show that absorbance values obtained with equal weights of sucrose and fructose are alike and slightly higher than those obtained with glucose. These data as well as those obtained with other sugars (Table 23, p.103) confirm the applicability of the anthrone procedure for TSS analysis. The anthrone procedure can also be used to determine NRD if the reactive reducing sugars are inactivated by alkaline enolization (Van Handel, 1968; Riazi et al., 1985) before the color reaction. Because conversion of free aldehyde or keto groups to corresponding alcohols should also change reducing sugars to anthrone unreactive forms, glucose, fructose, and sucrose were reacted with sodium and potassium borohydrides to see if an alternative method for determining NRD could be developed. The results (Table 17) show that enolizations or borohydride reductions eliminate reactions of glucose or fructose with anthrone, but color

Table 17. Enolization or borohydride reduction reactions of monosaccharides and sucrose and subsequent absorbance values obtained with anthrone reactions. -- Samples were enolized or reduced as outlined in the methods section, and absorbances obtained in reactions with anthrone were measured at 625 nm. Values are the means of 3 replications for KOH enolization and $NaBH_4$ reduction reactions, and a single determination for the $KBH_4$ reduction.

| Concentration (µg/Rx) | Absorbance Values for Reaction (Rx) Condition | | |
|---|---|---|---|
| | + KOH | + $NaBH_4$ | + $KBH_4$ |
| | | Sucrose | |
| 20 | 0.375 ± 0.01 | 0.399 ± 0.040 | 0.427 |
| 40 | 0.709 ± 0.04 | 0.733 ± 0.040 | 0.803 |
| 60 | 1.041 ± 0.04 | 1.102 ± 0.040 | 1.192 |
| 80 | 1.423 ± 0.04 | 1.472 ± 0.006 | 1.493 |
| | | Glucose | |
| 25 | 0.000 | 0.000 | 0.000 |
| 50 | 0.000 | 0.000 | 0.000 |
| 100 | 0.000 | 0.000 | 0.000 |
| | | Fructose | |
| 25 | 0.000 | 0.000 | 0.000 |
| 50 | 0.000 | 0.000 | 0.000 |
| 100 | 0.000 | 0.000 | 0.000 |

development with sucrose is unaffected. Although either of the borohydrides is effective, sodium borohydride is the preferred reagent due to its greater solubility in alcohol.

Studies aimed at speeding the hydrolysis of excessive borohydride with acetic acid fortuitously provided a means for selectively determining ketoses in the presence of aldoses. As a check to determine the effects of acetic acid on anthrone reactions, glucose, sucrose, and fructose were reacted at 97°C for 5 min in anthrone reagents that varied in the acetic acid/sulfuric acid ratios (Table 18). These studies showed (e.g., Column 1) that suitable high acetic acid:sulfuric acid ratios can virtually eliminate color development by glucose while retaining color formation with fructose or sucrose.

Although anthrone in acetic/sulfuric acid appears suitable for analyzing ketoses in leaf tissues, it was not applicable to pollen analysis because the characteristic green color associated with these reactions faded quickly to a dark yellow color. As a result, efforts were made to develop alternative analytical procedures.

## Selective Analysis of Keto Sugars

As Dische (1962) showed that carbohydrates could be analyzed with carbazole cysteine in sulfuric acid, trials were performed to see if a modification of the Dische procedure could be developed to determine keto sugars selectively. Such studies showed that in the presence of acetic acid/sulfuric acid mixtures (Table 19), fructose or sucrose were reactive and glucose was virtually unreactive with carbazole or carbazole cysteine. With carbazole, these keto sugars produced a brick

Table 18. Effects of varying sulfuric-acetic acid proportions on anthrone reactions with sucrose, fructose, and glucose. -- Specified amounts of sugars in 0.1 ml of 80% ethanol were reacted at 97°C for 5 min with 3 ml anthrone containing 180 mg anthrone in the indicated volumes of sulfuric-acetic acids. Absorbances were determined at 430 nm.

| Conc. (µg/Rx) | Absorbance Values for Reaction (Rx) Condition[a] | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | | | Sucrose | | | |
| 25 | 0.054 | 0.151 | 0.128 | 0.126 | 0.111 | 0.192 |
| 50 | 0.119 | 0.304 | 0.256 | 0.246 | 0.212 | 0.294 |
| 100 | 0.234 | 0.619 | 0.502 | 0.513 | 0.441 | 0.623 |
| | | | Fructose | | | |
| 25 | 0.117 | 0.259 | 0.233 | 0.212 | 0.166 | 0.193 |
| 50 | 0.221 | 0.518 | 0.464 | 0.426 | 0.340 | 0.333 |
| 100 | 0.460 | 1.065 | 0.917 | 0.737 | 0.675 | 0.654 |
| | | | Glucose | | | |
| 25 | 0.000 | 0.036 | 0.008 | 0.014 | 0.022 | 0.135 |
| 50 | 0.005 | 0.064 | 0.016 | 0.033 | 0.062 | 0.205 |
| 100 | 0.002 | 0.123 | 0.031 | 0.056 | 0.117 | 0.363 |

a. Reaction conditions:

1. 3 ml $H_2SO_4$ in 87 ml glacial acetic acid.
2. 5 ml $H_2SO_4$ in 85 ml glacial acetic acid.
3. 6 ml $H_2SO_4$ in 84 ml glacial acetic acid.
4. 10 ml $H_2SO_4$ in 80 ml glacial acetic acid.
5. 16 ml $H_2SO_4$ in 74 ml glacial acetic acid.
6. 30 ml $H_2SO_4$ in 60 ml glacial acetic acid.

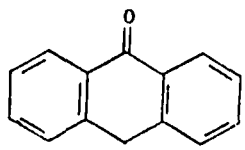
Table 19. Absorbance values following reactions of carbazole and carbazole-cysteine reagents with sugars. -- Sugars in 100 µl of 80% ethanol were reacted with 3 ml carbazole reagent (10 ml conc. $H_2SO_4$ in 90 ml glacial acetic acid containing 100 mg carbazole) and heated at 97°C for 15 min, cooled, and read at 490 nm or 3 ml carbazole-cysteine reagent (10 ml conc. $H_2SO_4$ in 90 ml glacial acetic acid containing 100 mg carbazole and 45 mg cysteine) and heated at 97°C for 4 min before reading at 690 nm. Rx indicates reaction.

| Concentration (µg/Rx) | Absorbance Values for Reagent | |
|---|---|---|
| | Carbazole | Carbazole-cysteine |
| | Sucrose | |
| 25 | 0.289 | 0.247 |
| 50 | 0.547 | 0.525 |
| 75 | 0.812 | 0.720 |
| 100 | 1.106 | 0.917 |
| | Fructose | |
| 25 | 0.490 | 0.472 |
| 50 | 0.943 | 0.839 |
| 75 | 1.377 | 1.063 |
| 100 | 1.839 | 1.403 |
| | Glucose | |
| 25 | 0.031 | 0.015 |
| 50 | 0.037 | 0.023 |
| 100 | 0.046 | 0.038 |

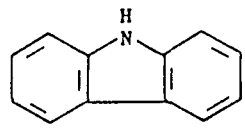
red color that absorbed maximally at 490 nm whereas the purple color formed in carbazole cysteine reactions absorbed maximally at 690 nm, and color fade did not occur with either reagent when mixtures were heated for up to 20 min at 97°C.

Color development in carbazole reagents, however, can be altered by alkali and perhaps other ions. When sucrose was analyzed in the presence of 1.8 N KOH, the concentration normally used for enolizing monosaccharides, the carbazole reagent yielded a purple rather than brick color; additionally, the colors produced in pollen extracts varied from brown to green to blue grey (data not shown). Possible ion effects on color development were confirmed by studies which showed that reaction mixtures containing 2 mg $Rb_2CO_3$, $K_2CO_3$, or KOH per 100 ml reagent led to the formation of purple rather than brick colored products, but absorbance values obtained were proportional to the concentrations of sucrose or fructose in solution (data not shown). The basis for the color change is unknown, but the results showed that although carbazole reagents produce a stable product and are potentially useful for analyzing keto sugars selectively, further studies must be performed to characterize ion effects before these substances are used to analyze carbohydrates in plant tissues, which are known to contain diverse ions.

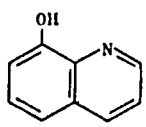
In order to understand more about the basis for color development of keto sugars with anthrone, carbazole, and similar compounds, studies were conducted with the constituent moieties of anthrone and carbazole and similar compounds (see below).



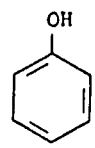

Anthrone



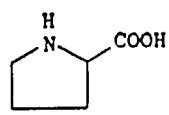

Carbazole




8-Hydroxyquinoline




Phenol




Proline

As expected, the sugars did not produce colored substances with proline, but sucrose and fructose reacted with the phenol-acetate reagent (Column 2, Table 20). Color development occurred within 5 min and the yellow color was stable for at least 25 min at 97°C (Fig. 23). This suggests that the ultimate color formed in anthrone and carbazole reactions may be due to the phenol moiety. Further, because the phenol-acetate reagent was unaffected by $K^+$ and consistently produced a yellow color in reactions with pollen extracts, it was considered the reagent of choice for determining keto sugars in both plant and pollen extracts.

A yellow color was also produced when the sugars were in the presence of 8-hydroxyquinoline (Column 3), but blanks that contained no

Table 20. Absorbance values following reactions of carbazole-Benedict, phenol, and 8-hydroxyquinoline reagents with sugars. -- Values represent absorbances at 495 nm, 440 nm, and 455 nm for carbazole-Benedict, phenol, and 8-hydroxyquinoline reagents, respectively.

| Conc. (µg/Rx) | Absorbance Values for Reaction (Rx) Conditions[a] | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| | | Sucrose | |
| 25 | 0.225 | 0.246 | 0.127 |
| 50 | 0.448 | 0.405 | 0.056 |
| 100 | 0.870 | 0.783 | 0.127 |
| | | Fructose | |
| 25 | 0.414 | 0.380 | 0.049 |
| 50 | 0.816 | 0.721 | 0.115 |
| 100 | 1.471 | 1.461 | 0.256 |
| | | Glucose | |
| 25 | 0.036 | 0.006 | 0.000 |
| 50 | 0.050 | 0.010 | 0.000 |
| 100 | 0.092 | 0.021 | 0.001 |

a. Reaction conditions:

1. Sugar standards in 100 µl with 80% ethanol were reacted with 3 ml carbazole-Benedict reagent (90 ml glacial acetic acid + 10 ml conc. $H_2SO_4$ + 100 mg carbazole + 5 ml Benedict reagent). A purple color developed. Absorbances obtained at 496 nm.

2. Sugar standards were same as in Reaction 1 and were reacted with 3 ml phenol reagent (53 ml glacial acetic acid + 6 ml conc. $H_2SO_4$ + 1 ml phenol containing 0.1% 8-hydroxyquinoline). Fructose and sucrose developed a yellow color. Absorbances obtained at 440 nm.

3. Sugar standards were reacted with 3 ml 8-hydroxyquinoline reagent (53 ml glacial acetic acid + 6 ml conc. $H_2SO_4$ + 50 mg 8-hydroxyquinoline). The blank was yellow and fructose and sucrose developed yellow coloration. Absorbances obtained at 455 nm.

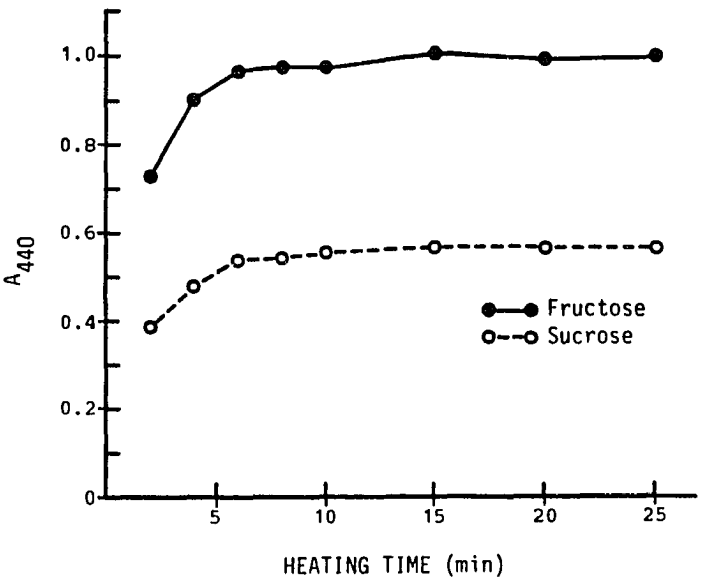



Figure 23. Reaction kinetics of sugars with phenol-acetate reagent. -- Sucrose and fructose standards (50 µg) in 100 µl 80% ethanol and reacted with 4 ml phenol-acetate reagent and heated at 97°C in boiling water for indicated heating period.

sugars also were colored. Because of the high backgrounds, further work was not done with this substance.

Livingston, Maurmeyer, and Worthan (1957) reported that keto sugars in acetic/sulfuric acid solutions yielded 4-hydroxymethyl furfural, but this is probably not the substance involved in color formation of keto sugars with phenol, carbazole, or anthrone for the following reasons:

1. In the absence of acetic acid, anthrone in sulfuric acid reacts with both ketoses and aldoses. The intermediate formed is 4-hydroxymethyl furfural (Newth, 1951), and the final product is green (Tables 15 and 16; also see Yemm and Willis, 1954).

2. In acetic/sulfuric acid, however, anthrone will form a colored product only with ketoses. Initially, anthrone will form a green color, but after 5 min at 97°C the final product is yellow (Table 18) and stable as is the reaction product with phenol-acetate (Table 20). Carbazole, however, yields a stable brick color (Table 19), but this color can be modified if alkali and other ions are present.

These results indicate the same basic mechanisms may be involved in all of the phenol-containing substances, but actual color developed may be altered by structural modifications not yet understood. The results also suggest that sugars containing keto groups can be specifically detected in the presence of aldo sugars because of modifications effected by acetic acid.

Although the phenol-acetate reagent was used extensively to analyze for ketoses in plant and pollen extracts, the TBA procedure developed by Percheron (1962, 1967) for identifying ketoses on TLC was also optimized for spectrophotometric analysis of ketoses in both plant and pollen extracts (Table 21). In these studies, absorbance values were determined using reagents that contained variable amounts of ethanol and HCl and the results show that this substance, as well as various phenolic compounds in acetic/sulfuric acid, can be used to quantitatively determine ketoses in the presence of aldoses. The application of the TBA procedure for identifying ketoses on TLC is being published (Arslan et al., n.d.).

Determinations of TRS

As the Nelson (1944) and Somogyi (1952) alkali cuprous ion procedures for determining reducing sugars can be affected by light and air

Table 21. Reaction conditions to optimize concentrations of HCl, ethanol, 2-thiobarbituric acid for ketose-specific reactions as measured by absorbance values. -- Absorbance values were obtained at 440 nm after reacting 3 ml indicated reagent with 0.1 ml sugar in 80% alcohol at 97°C for 20 min. TBA (150 mg) was added to indicated amounts of acidified ethanolic solutions.

| Concentration (µg/Rx) | Absorbance Values for Reaction (Rx) Condition[a] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | | Sucrose | | |
| 25 | 0.145 | 0.241 | 0.598 | 0.698 |
| 50 | 0.135 | 0.477 | 1.108 | 1.384 |
| 100 | 0.594 | 0.913 | 2.143 | 2.446 |
| | | Fructose | | |
| 25 | 0.283 | 0.449 | 1.013 | 1.269 |
| 50 | 0.540 | 0.879 | 2.017 | 2.296 |
| 100 | 1.125 | 1.686 | too dark | too dark |
| | | Glucose | | |
| 25 | 0.000 | 0.021 | 0.028 | 0.030 |
| 50 | 0.000 | 0.039 | 0.060 | 0.058 |
| 100 | 0.000 | 0.075 | 0.114 | 0.111 |

a. Reaction conditions:

1. 7 ml conc. HCl in 23 ml 10% ethanol.
2. 11 ml conc. HCl in 19 ml 10% ethanol.
3. TBA in 19 ml 25% ethanol was mixed with ethanolic HCl (11 ml conc. HCl mixed with 5 ml 100% ethanol).
4. TBA in 19 ml 25% ethanol was mixed with ethanolic HCl (11 ml conc. HCl mixed with 10 ml 100% ethanol).

(Dische, 1962), an alternative approach was sought to determine reducing sugars. Specifically, the alkaline p-HBAH procedure first developed by Russel and Lyons (1969) and used by Lever (1972, 1977) for analysis of glucose in blood was adapted for use in plant tissues. As was noted by Lever, the optimum concentration of p-HBAH for reactions was found to be 0.76 g/100 ml of alkaline solution, but turbidity problems were encountered when reactions were conducted with 2% NaOH in 80% ethanol. The turbidity problems were eliminated when reactions were conducted with 0.5% NaOH in 80% ethanol, or 0.5%, 1%, or 2% NaOH in 50% alcohol or distilled water (Table 22). Absorbance values involving equimolar amounts of glucose were identical to those obtained with fructose (Table 22) and the pH optimum was 12. The reaction is simple to perform, relatively nontoxic, insensitive to air, and the developed color is stable for several days. Also, no defatting or deproteinization was required (data not shown).

Determination of Aldoses

Aldoses such as glucose have been determined earlier in blood (Feteris, 1965) and plant extracts (Riazi et al., 1985) with o-toluidine. Additionally, procedures were developed here for determination of aldoses with o-ethylaniline (see Methods, p. 83), and results of reactions of o-toluidine and o-ethylaniline with glucose, fructose, and other sugars are presented in Table 23. The data show that absorbance values obtained with mannose and glucose are similar but somewhat lower than those obtained with galactose. Fructose, fructose bisphosphate (FBP), arabinose, sucrose, and melezitose (a nonreducing trisaccharide consisting of glucose-fructose-glucose) are unreactive with either reagent. Reactions with melibiose (a reducing disaccharide of glucose and galactose) appear to

Table 22. Reaction conditions to optimize concentrations of ethanol and NaOH for TRS-specific reactions with p-HBAH reagent as measured by absorbance values. -- Sugars in 0.1 ml 80% ethanol were reacted with 3 ml p-HBAH reagent containing 0.76 g p-HBAH in 100 ml alkaline reagents. Reactions 1-3 were at 70°C for 10 min; Reactions 4-6 were at 83°C for 10 min; and Reaction 7 was at 97°C for 5 min.

| | Absorbance Value for Reaction (Rx) Condition[a] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | | 4 | 5 | | 6 | | 7 |
| Conc. (µg/Rx) | 420 nm | 420 nm | 420 nm | 400 nm | 420 nm | 420 nm | 410 nm | 420 nm | 400 nm | 395 nm |
| | | | | | Glucose | | | | | |
| 25 | 0.494 | 0.567 | 0.445 | 0.731 | 0.787 | 0.825 | 1.094 | 0.605 | 1.035 | 0.771 |
| 50 | 0.993 | 1.198 | 0.903 | 1.483 | 1.648 | 1.441 | 2.012 | 1.107 | 1.979 | 1.507 |
| 100 | 1.892 | 2.129 | 1.749 | too dark | too dark | too dark | too dark | 2.003 | too dark | 2.532 |
| | | | | | Fructose | | | | | |
| 25 | 0.468 | 0.574 | 0.484 | 0.754 | 0.921 | 0.840 | 1.164 | 0.666 | 1.144 | 0.791 |
| 50 | 0.957 | 1.104 | 0.932 | 1.496 | 1.620 | 1.554 | 2.152 | 1.053 | 2.021 | 1.543 |
| 100 | 1.901 | 2.004 | 1.804 | too dark | too dark | too dark | too dark | 2.202 | too dark | 2.604 |
| | | | | | Glucose + Fructose | | | | | |
| 25 | 0.462 | 0.565 | 0.425 | 0.683 | 0.809 | 0.676 | 0.998 | 0.567 | 1.003 | 0.712 |
| 50 | 0.939 | 0.987 | 0.858 | 1.384 | 1.523 | 1.468 | 2.025 | 1.151 | 2.026 | 1.429 |
| 100 | 1.818 | 1.924 | 1.730 | 2.564 | too dark | 2.309 | too dark | 1.974 | too dark | 2.516 |

a. Reaction conditions:

1. 80% ethanol containing 2% NaOH.
2. 80% ethanol containing 1% NaOH.
3. 80% ethanol containing 0.5% NaOH.
4. 50% ethanol containing 2% NaOH.
5. 50% ethanol containing 1% NaOH.
6. 50% ethanol containing 0.5% NaOH.
7. Distilled water containing 0.5% NaOH.

Table 23. Reactions of monosaccharides, reducing and nonreducing disaccharides, and trisaccharides with indicated reagents as measured by absorbance values. -- Arabinose, xylose and ribose are pentoses; glucose, galactose, fructose, fructose bisphosphate, and mannose are hexoses; sucrose, maltose, and melibiose are disaccharides; melezitose and raffinose are trisaccharides.

| | Reagent | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anthrone | | Carbazole | | PASA | | TBA | | p-HBAH | o-toluidine | o-ethylaniline |
| | Absorbance Value for Reaction (Rx) | | | | | | | | | | |
| Conc. (µg/Rx) | TSS | TDS[a] | TKS[b] | Suc[c] | TKS | Suc[c] | TKS | Suc[c] | TRS | Aldoses | Aldoses |
| | | | | | | Arabinose | | | | | |
| 25 | 0.022 | 0.000 | 0.016 | 0.000 | ----- | ----- | ----- | ----- | 0.528 | 0.000 | 0.013 |
| 50 | 0.043 | 0.000 | 0.035 | 0.000 | ----- | ----- | ----- | ----- | 1.088 | 0.000 | 0.027 |
| 100 | 0.119 | 0.000 | 0.058 | 0.000 | ----- | ----- | ----- | ----- | 2.051 | 0.000 | 0.071 |
| | | | | | | Xylose | | | | | |
| 25 | 0.033 | 0.006 | 0.033 | 0.000 | ----- | ----- | ----- | ----- | 0.587 | 0.018 | 0.003 |
| 50 | 0.070 | 0.000 | 0.064 | 0.000 | ----- | ----- | ----- | ----- | 1.196 | 0.032 | 0.032 |
| 100 | 0.162 | 0.000 | 0.112 | 0.000 | ----- | ----- | ----- | ----- | 2.114 | 0.071 | 0.086 |
| | | | | | | Ribose | | | | | |
| 25 | 0.028 | 0.000 | 0.009 | 0.000 | ----- | ----- | ----- | ----- | 0.435 | 0.018 | 0.003 |
| 50 | 0.052 | 0.002 | 0.030 | 0.000 | ----- | ----- | ----- | ----- | 0.882 | 0.027 | 0.022 |
| 100 | 0.120 | 0.004 | 0.053 | 0.000 | ----- | ----- | ----- | ----- | 1.568 | 0.051 | 0.061 |
| | | | | | | Glucose | | | | | |
| 25 | 0.395 | 0.000 | 0.006 | 0.000 | 0.005 | 0.000 | 0.014 | 0.000 | 0.499 | 0.102 | 0.092 |
| 50 | 0.771 | 0.000 | 0.018 | 0.000 | 0.014 | 0.000 | 0.029 | 0.000 | 0.971 | 0.208 | 0.187 |
| 100 | 1.451 | 0.000 | 0.027 | 0.000 | 0.044 | 0.000 | 0.069 | 0.000 | 1.951 | 0.423 | 0.387 |

Table 23. -- Continued.

| Conc. (µg/Rx) | Reagent: Anthrone | | Carbazole | | PASA | | TBA | | p-HBAH | o-toluidine | o-ethylaniline |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Absorbance Value for Reaction (Rx) | | | | | | | | | | |
| | TSS | TDS[a] | TKS[b] | Suc[c] | TKS | Suc[c] | TKS | Suc[c] | TRS | Aldoses | Aldoses |
| | | | | | | Galactose | | | | | |
| 25 | 0.199 | 0.003 | 0.010 | 0.000 | ----- | ----- | ----- | ----- | 0.473 | 0.149 | 0.131 |
| 50 | 0.398 | 0.015 | 0.019 | 0.000 | ----- | ----- | ----- | ----- | 0.824 | 0.310 | 0.268 |
| 100 | 0.747 | 0.024 | 0.033 | 0.000 | ----- | ----- | ----- | ----- | 1.514 | 0.678 | 0.553 |
| | | | | | | Fructose | | | | | |
| 25 | 0.475 | 0.000 | 0.353 | 0.000 | 0.585 | 0.004 | 1.147 | 0.000 | 0.459 | 0.000 | 0.004 |
| 50 | 0.788 | 0.004 | 0.662 | 0.000 | 1.165 | 0.000 | 2.192 | 0.000 | 1.049 | 0.000 | 0.013 |
| 100 | 1.513 | 0.000 | 1.227 | 0.000 | 2.270 | 0.007 | >3.000 | 0.000 | 1.870 | 0.018 | 0.032 |
| | | | | | | Fructose Bisphosphate | | | | | |
| 25 | 0.161 | 0.002 | 0.074 | 0.013 | ----- | ----- | ----- | ----- | 0.114 | 0.000 | 0.000 |
| 50 | 0.280 | 0.004 | 0.168 | 0.019 | ----- | ----- | ----- | ----- | 0.223 | 0.000 | 0.000 |
| 100 | 0.570 | 0.006 | 0.318 | 0.033 | ----- | ----- | ----- | ----- | 0.535 | 0.000 | 0.000 |
| | | | | | | Mannose | | | | | |
| 25 | 0.200 | 0.000 | 0.006 | 0.052 | ----- | ----- | ----- | ----- | 0.482 | 0.097 | 0.102 |
| 50 | 0.319 | 0.000 | 0.018 | 0.027 | ----- | ----- | ----- | ----- | 0.895 | 0.201 | 0.187 |
| 100 | 0.623 | 0.007 | 0.037 | 0.053 | ----- | ----- | ----- | ----- | 1.713 | 0.432 | 0.377 |
| | | | | | | Sucrose | | | | | |
| 25 | 0.454 | 0.473 | 0.178 | 0.177 | 0.297 | 0.259 | 0.612 | 0.624 | 0.000 | 0.000 | 0.004 |
| 50 | 0.901 | 0.838 | 0.356 | 0.368 | 0.590 | 0.540 | 1.213 | 1.205 | 0.013 | 0.000 | 0.022 |
| 100 | 1.700 | 1.765 | 0.686 | 0.677 | 1.224 | 1.013 | 2.316 | 2.324 | 0.022 | 0.000 | 0.046 |

Table 23. -- Continued.

| Conc. (µg/Rx) | Reagent: Anthrone | | Carbazole | | PASA | | TBA | | p-HBAH | o-toluidine | o-ethylaniline |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Absorbance Value for Reaction (Rx) | | | | | | | | | | |
| | TSS | TDS[a] | TKS[b] | Suc[c] | TKS | Suc[c] | TKS | Suc[c] | TRS | Aldoses | Aldoses |
| | | | | | | Maltose | | | | | |
| 25 | 0.317 | 0.000 | 0.004 | 0.000 | ----- | 0.000 | 0.014 | 0.000 | 0.250 | 0.022 | 0.009 |
| 50 | 0.628 | 0.009 | 0.014 | 0.000 | ----- | 0.000 | 0.031 | 0.000 | 0.449 | 0.032 | 0.027 |
| 100 | 1.180 | 0.022 | 0.028 | 0.000 | ----- | 0.000 | 0.079 | 0.000 | 0.930 | 0.056 | 0.032 |
| | | | | | | Melibiose | | | | | |
| 25 | 0.250 | 0.065 | 0.000 | 0.000 | ----- | ----- | ----- | ----- | 0.425 | 0.092 | 0.086 |
| 50 | 0.552 | 0.131 | 0.010 | 0.000 | ----- | ----- | ----- | ----- | 0.881 | 0.187 | 0.181 |
| 100 | 1.069 | 0.205 | 0.019 | 0.000 | ----- | ----- | ----- | ----- | 1.720 | 0.357 | 0.367 |
| | | | | | | Melezitose | | | | | |
| 25 | 0.370 | 0.418 | 0.089 | 0.116 | ----- | ----- | ----- | ----- | 0.013 | 0.000 | 0.000 |
| 50 | 0.692 | 0.810 | 0.175 | 0.218 | ----- | ----- | ----- | ----- | 0.028 | 0.000 | 0.004 |
| 100 | 1.309 | 1.456 | 0.329 | 0.422 | ----- | ----- | ----- | ----- | 0.042 | 0.000 | 0.009 |
| | | | | | | Raffinose | | | | | |
| 25 | 0.281 | 0.320 | 0.100 | 0.115 | ----- | ----- | ----- | ----- | 0.027 | 0.013 | 0.009 |
| 50 | 0.581 | 0.645 | 0.202 | 0.241 | ----- | ----- | ----- | ----- | 0.031 | 0.022 | 0.027 |
| 100 | 1.027 | 1.131 | 0.375 | 0.375 | ----- | ----- | ----- | ----- | 0.021 | 0.027 | 0.036 |

a. Glucose and fructose moieties of disaccharides were determined after alkaline elimination of monosaccharides.
b. Fructose and fructose moieties of disaccharides were determined.
c. Color formation of fructose moiety of disaccharides after alkaline elimination or reducing sugars.

yield absorbance values that are less than with either sugar alone. Slight color formations were also found with raffinose (a nonreducing sugar consisting of galactose, glucose, and fructose), maltose (a reducing glucose-glucose disaccharide), and also with the pentoses, xylose and ribose. It is presently unclear why the dissacharides melibiose and maltose differ so much in their reactivities, but results do show a selectivity of o-toluidine and o-ethylaniline for aldohexoses.

### Summarized View of Colorimetric Reactions with Sugars

As reactions are not totally specific, spectrophotometric reactions should be used in conjunction with physical or enzymatic procedures that provide alternative bases for understanding the sugar contents of plant tissues. For example, o-toluidine and o-ethylaniline will detect galactose as well as glucose, but Baker and Baker (1979) have shown through PC results that glucose, fructose, and sucrose are the major free sugars present in most plant tissues so the use of the above reagents will provide a good estimate of glucose within cells. Cell walls, however, can contain substantial amounts of galactose and pentoses as well as glucose, and analysis of wall hydrolysates will reflect reactivities due to galactose as well as glucose. Thus, if a tissue is analyzed for the first time, procedures such as PC and GLC should be employed as part of the process for characterizing free sugars, but once a general characterization is made, spectrophotometric methods will often provide rapid and convenient assays for many sugars.

The reactivities of various sugars with the reagents used in this study are summarized in Table 23 and an examination of the results shown, together with other considerations such as those noted above, provides a rational basis for using the reactions.

TSS results with anthrone show pronounced color formation with all hexoses or oligosaccharides of hexoses, but absorbance values can differ over 2-fold for different sugars. Color formation by pentoses is substantially less than with hexoses, but some reactivity does exist. If the same sugars are enolized with alkali prior to reaction with anthrone and analyzed for NRD, all reducing sugars examined (with exception of melibiose) are eliminated prior to reaction with anthrone. It is presently unclear why about 1/4 to 1/5 of the absorbance of melibiose is retained following enolization, but levels of melibiose are usually low in plant tissues. In general, the anthrone reagent is often usable for total carbohydrate analysis of plant tissues, and when the reaction is preceded by enolization or borohydride reduction to remove reducing sugars, it is also a convenient method for determining NRD. The enolization step has virtually no affect on the absorbance values obtained with trehalose (Arslan et al., 1986), sucrose, or the nonreducing trisaccharides, melezitose and raffinose (Table 23). The anthrone reaction, however, is affected by presently unknown substances present in pollen extracts.

Carbazole provides a much more stable color at higher temperatures than does anthrone and serves as a means for determining keto sugars (Column 3, Table 23). Some color development does occur with the pentoses arabinose, ribose, and xylose, but reactions with aldohexoses

such as glucose, galactose, mannose, etc. are virutally nonexistent. For a given weight of sugar, absorbance values of fructose were 2 times those of sucrose and 3 times those of melezitose or raffinose, which are the values expected based on the fructose contents of the oligosaccharides. FBP, however, was about 1/4 as reactive as free fructose with carbazole. Studies with phenol-acetate (Column 5, Table 23) and TBA (Column 7, Table 23) were less extensive than those with carbazole or anthrone, but as expected, the available data show that absorbances obtained with equal weights of sucrose were half those obtained with fructose. The phenol-acetate reagent is far less affected by ions than the carbazole reagent and is the one that probably can be developed further. Additional studies of other sugars with phenol acetate and TBA, however, must be made.

TRS determinations with p-HBAH under alkaline conditions showed that sucrose and the nonreducing trisaccharides, melezitose and raffinose, were virtually unreactive, whereas all hexoses and pentoses did react. The absorbance values obtained with equal weights of the different hexoses and pentoses varied somewhat more than expected on the basis of their molecular weights, and the existence of this variability may be why absorbance values obtained with the pentoses were not significantly higher than absorbance values obtained with the pentoses were not significantly higher than those obtained with the hexoses. Differences in absorbance values were obtained in reactions with reducing dissacharides. Both hexoses of melibiose ([6-(α-D-galactopyranosyl)-D-glucopyranose] were reactive with p-HBAH, whereas only one of the glucose units of

maltose [4-(α-D-glucopyranosyl)-D-glucopyranosel] reacted with the reagent. This is probably because of protection conferred by the α1,4 linkages under alkaline conditions. As in reactions with carbazole, the presence of phosphate residues on fructose also reduced the reactivity of this sugar with p-HBAH.

TSS Determinations with p-HBAH Reagent

In order to further characterize the nature of reactants in the p-HBAH reaction, a series of oxidations and methylations were performed with sucrose, glucose, and fructose, and the studies eventually led to a development of a procedure that permitted the analysis of TSS with this reagent.

When compared with p-HBAH reactions performed in the absence of oxidizing or methylating agents (Table 23), prior periodate treatment dramatically increased color development by sucrose but reduced absorbance values obtained with fructose or glucose (Column 1, Table 24). Although the reasons for these effects are not known, such results indicated further studies with periodate oxidations may be futile. On the other hand, oxidation with aqueous 2% $KMnO_4$ (Column 2) or with Baeyer's alkaline permanganate (Shriner, Fuson, and Curtin (1964); Column 3) prior to p-HBAH did not affect the absorbance values obtained with the monosaccharides but slightly increased absorbances obtained with sucrose. Methylations of sugars (Migridichian, 1957) with absolute methanol containing 1% concentration HCl (Column 4) slightly increased reactivity of sucrose with p-HBAH but tended to reduce absorption values obtained with fructose or glucose; the use of methanol containing 10% HCl

Table 24. Effects of prior periodate or permanganate oxidation or methylation on reactivity of sugars with p-HBAH as measured by absorbance values. -- Sugar standards in 0.1 ml 80% ethanol were mixed with indicated amounts of oxidizing or methylating agents at 26°C, then reacted with 4 ml p-HBAH for 10 min at 70°C and read at 420 nm.

| Concentation (µg/ml) | Absorbance Values for Reaction (Rx) Condition[a] | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 |
| | | Sucrose | | | |
| 25 | 0.266 | 0.076 | 0.099 | 0.153 | 0.094 |
| 50 | 0.420 | 0.127 | 0.058 | 0.463 | 0.177 |
| 100 | 1.022 | 0.161 | 0.183 | 1.534 | 0.321 |
| | | Fructose | | | |
| 25 | 0.191 | 0.537 | 0.530 | 0.092 | 0.392 |
| 50 | 0.345 | 1.128 | 1.045 | 0.364 | 0.783 |
| 100 | 0.744 | 1.955 | 1.967 | 1.328 | 1.626 |
| | | Glucose | | | |
| 25 | 0.150 | 0.823 | 0.599 | 0.196 | 0.444 |
| 50 | 0.394 | 1.401 | 1.362 | 0.363 | 0.882 |
| 100 | 0.760 | 2.252 | 2.317 | 1.406 | 1.755 |

a. Reaction conditions:

1. Periodic acid (200 µl, 0.3 M).
2. $KMnO_4$ (1 µl, 2%).
3. $KMnO_4$ (2 µl, 2%), then followed with 50 µl 0.1 N NaOH (Baeyer's test).
4. Methanolic HCl (100 µl of 1% = 1 ml conc. HCl + 99 ml absolute methanol).
5. Methanolic HCl (100 µl of 10% = 10 ml conc. HCl + 90 ml absolute methanol).

on the other hand, tended to retain the reactivity of glucose and fructose with p-HBAH, but absorbance values obtained with sucrose were lower than those resulting from reactions with 1% HCl.

Because the above reactions offered the potential for obtaining absorbance values for sucrose as well as glucose and fructose, various combinations of permanganate oxidations or methylations followed by permanganate oxidations were performed to determine if sucrose as well as the hexoses could be analyzed with p-HBAH (Table 25). The data show that sugar standards that are treated first with 25 µl 10% HCl in methanol and subsequently followed by oxidation with 5 µl of 2% $KMnO_4$ will optimally enhance the reactivity of sucrose while affecting hexoses minimally (Column 2, Table 25). This provides an alternative procedure for analyzing for TSS, and as such offers a check for data obtained with anthrone. Because p-HBAH permits color formation with pentoses but anthrone does not (Table 23), the hydrazide reagent should provide a better estimate of TSS.

A limited number of comparisons of TSS data obtained with anthrone and p-HBAH have been performed with extracts from plants and studies with extracts of growing regions of both stressed and unstressed barley leaves have shown the techniques provide virtually identical results. In one study, replicated 4 times, unstressed plants had 26.12 + 1.03% TSS with the anthrone method and 24.94 + 1.61% TSS with the p-HBAH procedure; the respective values for tissues stressed for 4 hr in -8 bars PEG were 26.14 + 0.72% and 23.92 + 1.61%.

Table 25. Effects of methylation and permanganate oxidation of mixtures of sugars as measured by absorbance values. -- Sugars in 0.1 ml 80% ethanol were mixed with indicated amounts of methanolic HCl and potassium permanganate at 26°C, then reacted with 3 ml p-HBAH at 70°C for 10 min and read at 420 nm.

| Concentration (µg/ml) | Absorbance Values for Reaction Condition[a] | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | | Sucrose | | |
| 25 | 0.297 | 0.609 | 0.741 | 0.634 |
| 50 | 0.578 | 1.153 | 1.357 | 1.368 |
| 100 | 1.011 | 1.965 | 2.316 | 2.179 |
| | | Fructose | | |
| 25 | 0.497 | 0.481 | 0.485 | 0.490 |
| 50 | 0.968 | 0.882 | 0.973 | 0.957 |
| 100 | 1.468 | 1.523 | 1.759 | 1.803 |
| | | Glucose | | |
| 25 | 0.990 | 0.513 | 0.808 | 0.663 |
| 50 | 1.550 | 1.110 | 1.495 | 1.290 |
| 100 | 2.052 | 1.922 | 2.360 | 2.093 |
| | | Sucrose + Glucose + Fructose | | |
| 25 | 0.711 | 0.502 | 0.748 | 0.585 |
| 50 | 1.189 | 1.015 | 1.341 | 1.154 |
| 100 | 1.652 | 1.882 | 2.111 | 2.078 |

a. Reaction conditions:

1. Methanolic HCl (100 µl, 10%) followed by 2 µl 2% $KMnO_4$
2. Methanolic HCl (100 µl, 10%) followed by 5 µl 2% $KMnO_4$
3. Methanolic HCl (25 µl, 10%) followed by 5 µl 2% $KMnO_4$
4. Methanolic HCl (20 µl, 10%) followed by 5 µl 2% $KMnO_4$

## Analysis of Soluble Carbohydrates in Pollen

An examination of the literature shows that only a single attempt (Todd and Bretherick, 1942) has been made to screen the soluble and insoluble carbohydrates found in pollen from different plant species, but several others (e.g., Watanabe, Motomura, and Aso, 1961) have attempted to determine carbohydrates in pollen from specific plants. The technique used by Todd and Bretherick was not stated, but Watanabe et al. collected large quantities of Typha pollen and separated sugars with PC, then quantified the levels of each spot spectrophotometrically and further crystallized the contents of the major spots and identified the crystals microscopically. The work of Watanabe et al. was carefully done, but was extremely tedious.

As the carbohydrates that occur in pollen during anthesis may affect viability and attractiveness to insects, a specific effort was made to identify soluble carbohydrates in pollens of various species. Thus far, more than 100 species have been examined.

It was immediately evident that pollen extracts made with 80% alcohol contained residues which clogged HPLC columns and prevented carbohydrate analysis. These residues appeared to be phenolic glycosides which prevented suitable derivatization of free sugars for GLC. For these and other reasons, attempts were made to analyze the carbohydrates spectrophotometrically, but the soluble phenolic glycosides (based on spot tests according to Harborne (1981)) also were found to often interfere with free soluble sugar determinations. As a result, prior separation of pollen extracts was used to remove interfering substances.

Comparative data obtained from analysis of direct extracts and extracts partially purified through columns (Tables 26 and 27) show direct alcohol extracts had about 50%-70% higher TSS values than column purified extracts (Column 1, Table 26); additionally, examination of the direct extracts show values of component sugars usually exceeded the TSS values. For example, in the case of pollen from Juglans regia, TRS (Column 4, Table 26) + NRD (Column 1 or 2, Table 27) = 7.4 + 8.9 = 16.3% is larger than the 12.6% obtained for TSS (Column 1, Table 26). In contrast, similar calculations of TRS + NRD of the column purified substances show the sums were the same as the TSS values. Also, determinations of TKS by phenol-acetate reagent and TBA (Columns 2 and 3, Table 26) and sucrose after KOH enolization or borohydride reduction (Columns 3-6, Table 27) were usually alike for column eluates but not for the direct extracts. Such results, together with other data showing individual sugars were not retained by the columns, suggested column purifications were required to eliminate interfering substances, and the prior purification step was routinely incorporated in further studies with pollens. It should be noted, however, attempts to analyze sucrose following KOH enolization of eluates (Column 3, Table 27) led to significant absorbances which were unrelated to the concentrations of sucrose present in extracts. This presently unexplained phenomenon, however, did not occur following borohydride reductions of eluates (Column 4).

Comparisons of the soluble sugars and starches in pollen from Typha compared favorably to those obtained by Watanabe et al. (1961)

Table 26. Sugar content of pollen samples determined by different reactions after ethanol extraction and following column elution. -- Values are means ± SD of 3 replications, except for those footnoted "a" are mean value of duplicate determinations.

| Taxa | Reagent: Anthrone | PASA | TBA | p-HBAH | o-toluidine |
|---|---|---|---|---|---|
| | Reaction: TSS % | TKS % | TKS % | TRS % | Glucose % |
| | **Alcohol Extract** | | | | |
| Cynadon dactylon | 15.07 ± 0.20 | 13.30 ± 0.33 | 10.31 ± 0.02 | 18.24 ± 0.17 | 5.96 ± 0.08 |
| Juglans regia calif. | 12.62 ± 0.45 | 14.80 ± 0.07 | 13.85 ± 0.56 | 7.43 ± 0.11 | 4.26 ± 0.65 |
| Rumex crispus | 19.96 ± 0.39 | 13.03 ± 0.19 | 11.69 ± 0.33 | 17.13 ± 0.36 | 8.55 ± 1.01 |
| Typha latifolia | 20.05 ± 0.06 | 13.65 ± 0.24 | 10.46 ± 0.46 | 20.47 ± 0.18 | 12.08 ± 0.80 |
| Phoenix dactylifera | 17.66 ± 0.19 | 13.88 ± 0.03 | 10.59 ± 0.18 | 18.19 ± 1.28 | 8.96 ± 1.15 |
| Poa annua | 16.91 ± 0.35 | 15.34 ± 0.01 | 11.95 ± 0.21 | 15.87 ± 1.10 | 7.12 ± 0.63 |
| Zea mays | 12.47 ± 0.21 | 9.97 ± 0.34 | 6.29 ± 0.37 | 12.03 ± 0.22 | 5.96 ± 0.22 |
| Typha sp. | 20.69 ± 0.33 | 13.42 ± 0.00 | 9.91 ± 0.33 | 18.65 ± 0.85 | 10.00 ± 0.45 |
| | **Column Eluates** | | | | |
| Cynadon dactylon | 7.79 ± 0.04 | 5.77 ± 0.12 | 5.96 ± 0.09 | 8.19 ± 0.04 | 2.35[a] |
| Juglans regia calif. | 6.32 ± 0.19 | 4.13 ± 0.00 | 4.29 ± 0.18 | 2.43 ± 0.42 | 1.46 ± 0.25 |
| Rumex crispus | 10.62 ± 0.78 | 5.55 ± 0.03 | 5.91 ± 0.12 | 7.46 ± 0.54 | 4.16 ± 1.02 |
| Typha latifolia | 9.18 ± 0.18 | 4.93 ± 0.09 | 5.10 ± 0.11 | 9.19 ± 1.08 | 3.82 ± 0.60 |
| Phoenix dactylifera | 8.19 ± 0.45 | 4.91 ± 0.02 | 5.06 ± 0.15 | 5.71 ± 0.57 | 3.11 ± 0.87 |
| Poa annua | 7.64 ± 0.16 | 4.92 ± 0.22 | 4.96 ± 0.14 | 6.13 ± 0.46 | 3.08 ± 0.31 |
| Zea mays | 4.98 ± 0.32 | 2.69 ± 0.11 | 2.91 ± 0.11 | 3.80 ± 0.06 | 2.01 ± 0.31 |
| Typha sp. | 8.75 ± 0.91 | 4.37 ± 0.17 | 4.84 ± 0.12 | 8.05 ± 0.23 | 4.67[a] |

Table 27. NRD and sucrose contents of pollen estimated with different reagents. -- Values are means ± SD of 3 replications, except those footnoted "a".

| Taxa | Disaccharide (Anthrone) | | Sucrose (phenol-acetate) | | Sucrose (TBA) | |
|---|---|---|---|---|---|---|
| | KOH (5.4 N) (%) | $NaBH_4$ (.26 M) (%) | KOH (1.8 N) (%) | $NaBH_4$ (.26 M) (%) | KOH (5.4 N) (%) | $NaBH_4$ (.26 M) (%) |
| | | | Alcohol Extract | | | |
| Cynadon dactylon | 1.19 ± 0.01 | 1.31 ± 0.01 | 1.65 ± 0.16 | 2.13 ± 0.52 | 1.13 ± 0.22 | 1.19[a] |
| Juglans regia | 8.76 ± 0.49 | 8.96 ± 0.04 | 10.76 ± 0.37 | 13.16 ± 1.76 | 8.88 ± 0.34 | 9.29 ± 0.08 |
| Rumex crispus | 4.66 ± 0.13 | 4.54 ± 0.07 | 2.98 ± 0.73 | 3.82 ± 0.14 | 1.58 ± 0.06 | 1.06 ± 0.47 |
| Typha latifolia | 2.64 ± 0.15 | 1.99 ± 0.04 | 2.60 ± 0.04 | 3.89 ± 0.42 | 1.38 ± 0.11 | 0.44[a] |
| Phoenix dactylifera | 2.04 ± 0.02 | 1.96 ± 0.09 | 3.61 ± 0.84 | 4.32 ± 0.28 | 1.29 ± 0.03 | 1.18 ± 0.85 |
| Poa annua | 4.73 ± 0.12 | 4.71 ± 0.09 | 5.27 ± 0.44 | 6.28 ± 0.47 | 3.95 ± 0.07 | 3.39 ± 0.82 |
| Zea mays | 4.12 ± 0.04 | 3.66 ± 0.28 | 5.68 ± 4.53 | 3.95 ± 0.16 | 1.83 ± 0.06 | 1.68 ± 0.25 |
| Typha sp. | 3.05 ± 0.17 | 2.87 ± 0.08 | 3.95 ± 0.09 | 4.27 ± 0.62 | 1.24 ± 0.11 | 0.42 ± 0.20 |
| | | | Column Eluates | | | |
| Cynadon dactylon | 0.00 | 0.00 | (b) | 0.00 | 0.00 | 0.00 |
| Juglans regia | 4.29 ± 0.04 | 4.00 ± 0.08 | --- | 3.54 ± 0.00 | 3.94 ± 0.17 | 3.22 ± 0.21 |
| Rumex crispus | 2.57 ± 0.18 | 2.77 ± 0.44 | --- | 1.69 ± 0.05 | 1.86 ± 0.08 | 1.48 ± 0.45 |
| Typha latifolia | 0.35 ± 0.01 | 0.30 ± 0.01 | --- | 0.66 ± 0.41 | 0.46 ± 0.04 | 0.32[a] |
| Phoenix dactylifera | 2.41 ± 0.21 | 2.14 ± 0.06 | --- | 2.71 ± 0.06 | 2.83 ± 0.004 | 2.86 ± 0.11 |
| Poa annua | 1.74 ± 0.15 | 1.88 ± 0.28 | --- | 1.54 ± 0.02 | 1.82 ± 0.04 | 1.55 ± 0.33 |
| Zea mays | 0.78 ± 0.23 | 1.11 ± 0.09 | --- | 0.99 ± 31 | 1.20 ± 0.09 | 0.91 ± 0.28 |
| Typha sp. | 0.00 | 0.00 | --- | 0.00 | 0.00 | 0.00 |

a. Values are means ± SD of 2 replications.
b. Estimates of sucrose from column eluates following KOH enolization steps yielded absorbance values unrelated to color intensity.

(Table 28). The starch contents obtained with pollen from various species were similar to those obtained by Todd and Bretherick (1942) (Column 6, Table 28), but soluble carbohydrates were substantially lower. Because the values for soluble carbohydrates were similar to those obtained with the direct extracts prior to column purification, it is suggested that the higher values obtained by Todd and Bretherick were largely due to interfering glycosidic substances.

Starch and Glycogen Analysis

Although soluble and insoluble starches and glycogen have been analyzed for many years, many of the procedures are lengthy, or various factors may prevent accurate quantitative estimations of these substances. Starch has been analyzed by perchloric acid oxidation followed by determination of products with anthrone (e.g., McCready et al., 1950), but the procedure is lengthy and requires special hoods for oxidations. Alternatively, it has been hydrolyzed enzymatically and the glucose formed has been determined with glucose oxidase (Varns and Sowokinos, 1974). The procedure requires complete hydrolysis, and it has been reported that hydrolysis of glycogen is incomplete unless hydrolysis is conducted for 2 hr at 45°C (Kepler and Decker, 1977); additionally, the activity of the glucose oxidase assay is variable and requires fairly restrictive conditions (Fales et al., 1961). Because of the existence of these problems, and also because assays for starch in potato tubers were initially highly variable, a considerable effort was made to develop a rapid, reproducible procedure that provided a highly quantitative estimate of starch in potatoes and plant tissues, and glycogen in bees.

Table 28. Values for soluble sugars and starch in pollen determined in this study and by others

| Taxa | TSS (%) | Sucrose (%) | Glucose (%) | Fructose (%) | TRS (%) | Starch | | References |
|---|---|---|---|---|---|---|---|---|
| | | | | | | o-toluidine (%) | p-HBAH (%) | |
| Zea mays | 4.98 | 1.03 | 2.01 | 1.77 | 3.80 | 16.89 | 20.00 | 3 |
| Zea mays | 14.19 | 7.31 | --- | --- | 6.88 | 22.40 | | 1 |
| Typha latifolia | 9.18 | 0.50 | 3.82 | 4.52 | 9.19 | 15.93 | 18.53 | 3 |
| Typha latifolia | 18.92 | 18.88 | --- | --- | 0.04 | 13.01 | | 1 |
| Typha latifolia L. | 7.70 | 0.46 | --- | --- | 6.68 | 13.46 | | 2 |
| Typha sp. | 8.75 | 0.00 | 4.67 | 4.61 | 8.05 | 15.23 | 14,92 | 3 |
| Phoenix dactylifera | 8.19 | 2.80 | 3.11 | 2.19 | 5.71 | 0.43 | 1.88 | 3 |
| Phoenix dactylifera | 1.20 | 0.13 | --- | --- | 1.07 | 0.00 | | 1 |
| Cynadon dactylon | 7.79 | 0.00 | 2.35 | 5.87 | 8.19 | 10.53 | 12.83 | 3 |
| Cynadon dactylon | 28.96 | 3.43 | --- | --- | 25.53 | 0.37 | | 1 |

1. Calculated from Todd and Bretherick (1942; Table 3, p. 315).

2. Watanabe, Motomura, and Aso (1961, Table 1, p. 174).

3. This dissertation.

Initial studies with α-amyloglucosidase hydrolysis of pure potato starch in which incubations were conducted for 1 hr at 30°C in acetate buffer (pH 4.6) containing 1 mg enzyme/ml with the Varns and Sowokinos (1974) method showed that glucose equivalents determined with the o-toluidine procedure decreased with increasing sample size. When approximately 13 mg starch were hydrolyzed enzymatically, starch content was estimated as being 92%; however, recovery using 25 mg was oly 78%, and when 50 mg were used, percentages dropped to 58%. Elevating the temperature to 45°C and extending the hydrolysis period to 2 hr as suggested by Kepler and Decker (1977) led to 97% recovery when 15 mg starch were hydrolyzed enzymatically. When 45 mg were used, however, recoveries fell to 80% (data not shown).

The problems were resolved when starch and plant samples were first suspended in dilute alkali, neutralized, then enzymatically hydrolyzed with α-amyloglucosidase for 3-4 hr at 45°C. Results (Table 29) show that virtually complete recoveries of glucose residues can be obtained from starch samples up to about 0.05 g in size. Reactions with either anthrone, o-toluidine, or p-HBAH provided suitable estimates of the glucose residues. Because of its relative simplicity and lack of toxicity, and also because its sensitivity is about 3 times that of anthrone and 10 times that of o-toluidine, p-HBAH is considered the reagent of choice.

Table 29. Recovery of glucose equivalents in different-sized samples of pure potato starch. -- Starch was suspended in 5 ml N NaOH, neutralized and 1 ml aliquot of suspension was later hydrolyzed with 1 ml (1 mg/ml) of α-amyloglucosidase for indicated periods at 45°C. Glucose was then estimated with either anthrone, o-toluidine, or p-HBAH.

| Sample Size (g) | Hydrolysis Period (hr) | Estimated Percent Starch | | |
|---|---|---|---|---|
| | | Anthrone | o-toluidine | p-HBAH |
| 0.0118 | 3 | 95.7 | 98.9 | |
| 0.0197 | 3 | 103.6 | 97.8 | |
| 0.0365 | 3 | 97.1 | 98.7 | |
| 0.0316 | 4 | | 99.2 ± 2.8 | 98.11 ± 1.2 |
| 9.0312 | 4 | | 95.2 ± 2.3 | 96.6 ± 1.4 |

## TLC Analysis of Plant Tissue and Pollen Extracts

TLC separations of plant tissue extracts with B:A:W (Table 30) and DMF:B:W (Table 31) show that each of the extracts have a variety of sugars or sugar derivatives, and many of the spots are not coincident with the retention factor (rf) values of standard sugars commonly obtained from plant tissues, most of which lie between rf 0.1 to 0.36 in B:A:W and 0.42 to 0.65 in DMF:B:W (see bottom of tables). Both solvent systems tend to have higher rf values for less polar compounds, and other characterization reactions have shown that materials with rf values of 0.12, and 0.53 to 0.68 in B:A:W and 0.73 to 0.82 in DMF:B:W are glycosides. The substances found at rf 0.83 in B:A:W and 0.87 in DMF:B:W are chlorophylls, but the identity of the rf 0.93 substance in both TLC systems is unknown. Glycosides characteristically either fluoresce or absorb ultraviolet light of 360 and 254 nm and they also have spots that show color reactions with sugar reagents (Harborne, 1984); in contrast, free sugars do not absorb or fluoresce ultraviolet light. Chlorophylls characteristically give a deep red color under 360 nm ultraviolet light.

The basal region of barley leaves also has a variety of unidentified compounds (rf 0.00 to 0.10 in B:A:W; rf 0.00 to 0.53 in DMF:B:W) that have functional keto or aldehyde moieties, but keto compounds are dominant (based on visual color development of spot reactions; data not shown). The color reactions also showed that the intensities of these spots were higher than those of the free sugars, and stress for 4 hr at -8 bars increased their intensities even more. Such results indicate an effort should be made to identify these substances because their changes

Table 30. Rf values of leaf carbohydrates extracted with 80% ethanol, and sugar standards double run on TLC with B:A:W. -- Each value is the mean from 6 plates, and base and blade refer to extracts obtained from 1 cm sections from the leaf base and mid-blade, respectively, of 5-day-old barley seedlings. Mung bean leaves used for extraction were from 17-day-old seedlings. All plants were grown hydroponically, and barley seedlings were stressed with PEG 8000 (-8 bars, 4.5 hr).

| | Mean Rf Values of Spots | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | .00 | .02 | .04 | .07 | .10 | .12 | .14 | .19 | .23 | .31 | .33 | .36 | .53 | .56 | .63 | .68 | .83 | .93 |
| Base-control | .00 ±.00 | | .04 ±.01 | .07 ±.01 | .10 ±.01 | | .14 ±.01 | .18 ±.01 | .24 ±.01 | .31 ±.01 | .33 ±.01 | .36 ±.01 | | .56 | | | .81 | .93 ±.01 |
| Base-stressed | .00 ±.00 | .02 | .04 ±.01 | .07 ±.01 | .10 ±.01 | | .14 ±.01 | .18 ±.01 | .23 ±.02 | .31 | .34 ±.01 | .37 | | .56 ±.02 | .63 | | .82 | .91 ±.02 |
| Blade-control | | | | | | .12 | .14 ±.01 | .18 ±.01 | .23 ±.01 | .32 ±.02 | | | .53 ±.02 | | .63 ±.00 | | .82 ±.01 | .92 ±.01 |
| Blade-stressed | | | | | | .12 | .14 ±.01 | .19 ±.01 | .24 ±.02 | | .33 ±.02 | | .54 ±.03 | | .64 | | .82 | .91 ±.02 |
| Mung Bean (1st leaf) | | | | | | | | .19 | .23 ±.02 | .31 | .34 ±.02 | | .54 ±.01 | | .63 ±.02 | .65 | .84 ±.02 | .94 |
| Mung Bean (1st trifoliate) | | | | | | | | .19 | .23 ±.02 | .31 | .35 ±.02 | | | | .62 ±.01 | .65 | .83 ±.02 | .93 |
| Sugar Standards | inos galac stach | | | | raf | | mele meli | mal suc | suc | glc gal | fru glc | ara man fru | | | | | | |

Table 31. Rf values of leaf carbohydrates extracted with 80% ethanol, and sugar standards double run with DMF:B:W. -- Values are means of 6 plates for extracts used in Table 30.

| | Mean Rf Values of Spots | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample | .00 | .21 | .25 | .28 | .31 | .35 | .38 | .42 | .44 | .48 | .52 | .57 | .60 | .65 | .73 | .78 | .82 | .87 | .98 |
| Base-control | .00 ±.00 | .21 ±.01 | .25 ±.00 | .28 ±.01 | .30 ±.00 | .34 ±.01 | .38 ±.01 | .42 | .44 ±.01 | .48 ±.00 | .52 ±.02 | | .59 ±.00 | | | .77 .01 | | | .93 ±.01 |
| Base-stressed | .00 ±.00 | .21 ±.01 | .25 ±.01 | .28 ±.01 | .30 ±.00 | .33 ±.01 | .38 .01 | .42 | .44 ±.00 | .48 ±.01 | .53 | | .59 ±.00 | | | .77 ±.00 | .82 | .87 | .93 ±.01 |
| Blade-control | | | | | | | | | | | | | .59 ±.01 | | .73 | .76 | .83 ±.01 | .87 | .93 |
| Blade-stressed | | | | | | | | | | | | | .59 ±.01 | | .73 | .77 | .82 | .86 | .93 |
| Mung Bean (1st leaf) | | | | | | | | | | | | .56 | .59 | .65 | | .77 | .82 | .86 | .93 |
| Mung Bean (1st trifoliate) | | | | | | | | | | | | .57 | .59 | .65 | | .78 | .82 | .87 | .93 |
| Sugar Standards | inos | sorb | sorb | man | | | raf meli | | | | mele mal | suc gal | fru glc suc | man xyl ara | | | | | |

as well as those of sucrose and glucose (Riazi et al., 1985) are part of the stress response picture in barley leaves. Because their separations are effected much more effectively in DMF:B:W than in D:A:W, future studies on their identity should be done with the solvent containing DMF.

The low rf, unidentified carbohydrates are absent in the expanded blade regions of stressed or unstressed barley leaves and in mung bean leaves, and comparisons of spots show that contents of free sugars in the 2 groups of leaves differ somewhat. In addition to hexoses and sucrose found in both kinds of leaves, mung beans also have pentoses. Both TLC methods do not permit a clear resolution of many of the common sugars present in leaves (e.g., fructose from glucose), but further resolution can be made with the quantitative methods reported on in this dissertation and in other reports (e.g., Riazi et al., 1985).

TLC studies of pollen extracts showed many compounds that differed substantially from those found in plant leaf samples. The rf values of extracts of pollen from 8 species separated with DMF:B:W are shown in Table 32. These species had about 50% sucrose, fructose, and glucose (rf 0.57 to 0.62), but direct extracts had substantial amounts of glycosides (rf 0.00 to 0.55; also, rf 0.65 to 0.78, which appear to be phenolic glycosides) and also aglycones (rf 0.72 to 0.85) and some unidentified higher rf substances. When column eluates of alcoholic extracts were examined, only sucrose, fructose, and glucose were found except that pollen from Cynadon and Typha spp. did not contain sucrose. These results were supported by spectrophotometric studies that showed

Table 32. Rf values of pollen carbohydrates extracted with 80% ethanol and their column eluates following double run with DMF:B:W. -- Values are means from at least 4 plates.

| Taxa | | Mean Rf Values of Spots | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | .00 | .39 | .44 | .48 | .54 | .58 | .60 | .65 | .75 | .77 | .81 | .84 | .91 | .94 |
| Cynadon dactylon | eluate | | | | | | | .62 | | | | | | | |
| | extract | | .39 | | | .55 | | .62 | | .75 | .78 | | .84 | .92 | |
| Juglans regia calif. | eluate | | | | | | | .62 | | | | | | | |
| | extract | .00 | .39 | | | .54 | .57 | .60 | | | .77 | .80 | .85 | .91 | .95 |
| Rumex crispus | eluate | | | | | | | .60 | | | | | | | |
| | extract | | | .43 | .48 | .53 | .57 | .60 | | .72 | .77 | .79 | .84 | .92 | .95 |
| Typha latifolia | eluate | | | | | | | .61 | | | | | | | |
| | extract | | | .44 | .49 | .53 | | .61 | .65 | | .77 | .80 | .84 | .91 | .95 |
| Phoenix dactylifera | eluate | | | | | | .59 | .60 | | | | | | | |
| | extract | | | .43 | .49 | .53 | .58 | .60 | .65 | .75 | .76 | .80 | .84 | .92 | .95 |
| Poa annua | eluate | | | | | | .57 | .60 | | | | | | | |
| | extract | | | .43 | .49 | .54 | .58 | .61 | .65 | .75 | .77 | .80 | .84 | .92 | .95 |
| Zea mays | eluate | | | | | | .57 | .61 | | | | | | | |
| | extract | | | .44 | | .52 | .57 | .61 | | .74 | .77 | .81 | .84 | | .95 |
| Typha sp. | eluate | | | | | | | .60 | | | | | | | |
| | extract | | | | .49 | .54 | .58 | .62 | | | .78 | .81 | | | |

only pollen from Cynadon and Typha spp. did not contain sucrose (Table 28); additionally, the results from the spectrophotometric as well as other studies provided further evidence that the columns will separate free sugars from glycosides and other interfering sugar derivatives in pollen extracts.

## Summary and Conclusions

The major aim of this study was to refine and develop spectrophotometric methods for quantitatively determining the various carbohydrates present in plant tissues and pollen. In the course of this study, the effectiveness of a variety of reagents for determining the content of sugars found in plants was investigated and these investigations have led to the development of several new methods for estimating sugars and starches present in plants.

In my earliest studies, major difficulties were encountered when I attempted to determine TSS of plant tissues with the anthrone reagent. In the initial experiments, plant tissues were extracted with water and large variations in absorption values were obtained whenever attempts were made to analyze 30 or more samples. These variations occurred because the extracts did not disperse readily in the viscous anthrone reagent, and the problems were resolved by extracting plant tissues with 80% ethanol overnight. This extraction procedure was then used for all subsequent studies.

In an earlier study (Riazi et al., 1985), it was found that analysis of sucrose in the presence of KOH yielded absorbance values

with anthrone reactions which were about 20% higher than those expected on the basis of reactions with the constituent sugars without KOH. This enhancement occurred because of the presence of $K^+$, but a selected number of other ions (e.g., $Bi^{2+}$, $Rb^+$) effected similar increases in absorbance values, whereas $Cu^{2+}$ was without effect. Because absolute alcohol added to the anthrone reagent also enhanced color formation without ion effects, later reactions with this reagent were conducted in the presence of ethanol.

Addition of acetic acid to the anthrone-sulfuric acid reagent was found to cause a selective reaction with ketoses such as fructose and the fructose moiety of sucrose. However, the colored compound formed was found to be unstable, and a rapid transition from the normal green to a yellow color occurred by boiling for 5 min following color development. This led to studies of reactions of sugars with various compounds containing benzene rings and compounds that could be considered constituents of anthrone. Ketoses were found to react specifically with carbazole, phenol, and 8-hydroxyquinoline, and a stable color was produced in all cases. Carbazole produced a brick red color whereas phenol and 8-hydroxyquinoline yielded a yellow color similar to the decomposition product in the anthrone reaction. Because blanks also produced a yellow color with 8-hydroxyquinoline, no further studies were done with this reagent; additionally, as color formation by carbazole was affected by alkali ions, further development of reactions with the latter reagent were also suspended. However, as phenol did form a stable color that was unaffected by various ions, this reagent was developed as the

"phenol-acetate" procedure for determining keto sugars and sucrose in the presence of aldoses. Ketoses were also analyzed with a modification of the TBA procedure of Percheron (1962, 1967), which was used originally for identifying keto sugars on TLC. The phenol-acetate and TBA reactions both absorbed maximally at 440 nm, but equimolar amounts of ketoses yielded 1.9 times more absorbance with TBA.

Methods were also developed for determining nonreducing ketose containing sugars such as sucrose and raffinose in mixtures containing reducing sugars. This was effected by initially eliminating existing reducing sugars with either alkaline enolization or borohydride reduction and subsequent reaction with either phenol-acetate or TBA reagent. The borohydride reduction step can also be applied to determination of NRD or nonreducing trisaccharides with anthrone. Use of the phenol-acetate and TBA reagents in conjunction with alkali enolization or borohydride reductions provides a more selective means for determining nonreducing ketoses such as sucrose in mixtures of sugars.

TRS determinations by the Nelson and Somogyi (1952) procedure are very tedious and subject to several errors, therefore the p-HBAH procedure of Lever (1972, 1977) originally used for blood analysis was adapted for analysis of reducing sugars in plant and pollen samples. This required an optimization of ethanol and NaOH to eliminate turbidity, but the procedure finally developed was extremely simple, nontoxic, and equimolar amounts of glucose, fructose, and various pentoses yielded similar absorbances. This reagent is considered the one of choice for

determining reducing sugars in the presence of sucrose, trehalose, and other nonreducing sugars.

Glucose and other aldoses can be determined in plant tissues with o-toluidine reagent (Riazi et al., 1985). It was shown that o-ethylaniline is equally effective for determining aldohexoses in plant and pollen samples. The methods are straightforward and specific for aldohexoses, but both reagents are highly toxic and require special handling. In cases where alternative procedures are desired, aldoses can be determined by measuring the differences obtained for TRS determinations with p-HBAH and keto sugars (e.g., fructose) analyzed with either phenol-acetate or TBA reagent.

A series of oxidation and methylation reactions was conducted with sucrose, glucose, and fructose in order to determine TSS with the p-HBAH reagent. An effective procedure was developed by first methylating with methanolic HCl and subsequently oxidizing with potassium permanganate. Under these conditions, the reactivity of the hexoses to p-HBAH was unaffected but sucrose was hydrolyzed and made reactive, and the method does not require defatting or deproteinization. Because the p-HBAH reagent, unlike anthrone, reacts with pentoses, the new methylation/oxidation procedure is considered a better reagent for determining TSS in samples known to contain substantial amounts of pentoses. Comparisons of results with the modified p-HBAH procedure with those obtained with anthrone for extracts from plants, pollen, and bee feces yielded virtually identical results.

Detailed studies conducted with pollen from various studies showed that many contained glycosides which could constitute more than 50% of the total sugars present in extracts. Therefore, prior purification and removal of glycosides was performed with nonpolar cyclohexyl columns followed by strongly anionic exchange columns before determining free soluble sugars. The selective removal of the glycosides was confirmed by TLC. It is suggested that a similar procedure be applied to plant tissues if the presence of glycosides is suspected.

Starch was also determined quantitatively by suspending alcohol insoluble residues in dilute alkali, followed by rapid neutralization, and subsequent enzymatic hydrolysis with α-amyloglucosidase of the well-suspended material for at least 3 hr at 45°C. Glucose residues were determined either with anthrone, o-toluidine and p-HBAH, with the latter reagent being the one of choice because of its simplicity and nontoxic nature. Complete recoveries of starch were obtained in potato tubers, pollen, and bee feces.

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