UNIVERSITY OF SOUTHERN QUEENSLAND

STUDY OF TOTAL NON-STRUCTURAL CARBOHYDRATES' AND MACRONUTRIENTS INFLUENCE ON PECAN YIELDS

A Thesis submitted by

Miriam Villen Rodriguez

B.Eng.Sc.(Chemistry)

M.Eng.Sc. (Agriculture)

For the award of

Doctor of Philosophy

2022

ABSTRACT

Pecan yields vary substantially from one year to the next due to a number of environmental and management factors including the alternate bearing nature of the pecan tree. Carbohydrates are a direct product of photosynthetic activity, and it is accepted that the alternate bearing trait in pecans is directly correlated to the amount of carbohydrates synthesized and how these are distributed within the tree.

The objective of this work was to study (for the first time) the behaviour of carbohydrates and mineral reserves in different Australian pecan tree organs (leaves, trunk and exposed lateral roots) through the monthly measurement of total non-structural carbohydrates (TNSC), soluble carbohydrates and the macronutrients nitrogen (N), phosphorous (P) and potassium (K), with an emphasis on the role that these parameters played in yields.

The concentration of carbohydrates in the trunk steadily decreased from spring to early summer, and subsequently increased to a peak in autumn, then slowly declined during winter. A minimum was achieved in June, coinciding with the start of the Australian winter. The relationships between carbohydrates and yields were established for the months of September and October (late spring), and June of the current season (early dormancy), coinciding with early dormancy with subsequent yields. This is an important finding suggesting the potential use of carbohydrates in the trunk during winter and spring.

As indicated by the significant correlations obtained in spring and winter, this research demonstrates potential for the measurement of carbohydrates to become part of the monitoring regime to assess tree health and aid crop forecasting. This study has also highlighted the relationship between TNSC, nitrogen and phosphorous in leaves, with subsequent yields suggesting the use of leaf testing early in the season to assess nutrient status and yield potential. Further work to quantify the contribution of carbohydrate storage and nutritional status to yields is justified.

CERTIFICATION OF THESIS

I Miriam Estefania Villen Rodriguez declare that the PhD Thesis entitled Study of total non-structural carbohydrates' and macronutrients influence on pecan yields is not more than 100,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references, and footnotes. The thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Signed Miriam E Villen Rodriguez: Date:

Endorsed by:

John Billingsley
Principal Supervisor

Troy Jensen
Associate Supervisor

Richard Heerema
*Associat*e Supervisor

Rafael Rovirosa
Associate Supervisor

Student and supervisors' signatures of endorsement are held at the University.

This work is dedicated to my mother, Isabel Rodriguez Espinel, and the memory of my father, Juan Francisco Villen Sanchez.

"I am among those who think that science has great beauty ." Marie Curie

ACKNOWLEDGEMENTS

I would like to offer special thanks to my father who, although no longer with us, continues to inspire me in my personal life and career. Last, but not least, to my mother, Isabel; a courageous woman who taught my sisters and me to follow and fight for our dreams. Thank-you for always having the courage to face any adversity, and for moving ever forward regardless of the obstacles. You have taught me the best lessons in life.

TABLE OF CONTENTS

Page intentionally blank

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

TNSC   =   Total non-structural carbohydrates

SFE   =   Stahmann Farms Enterprises PTY LTD

HIA   =   Horticulture Innovation Australia Ltd

PT   =   Phytohormone Theory

CT   =   Carbohydrate Theory

GA   =   Gibberellic acid

CU   =   Chill units

HU   =   Heat hours

DPI   =   Department of Primary Industries

NIS   =   Nut in shell

T   =   Tones

ha   =   Hectares

HAl   =   Horticulture Australia Limited

BoM   =   Bureau of Meteorology

HCO   =   Hand Crack Out

CHAPTER 1 : INTRODUCTION

1.1 BACKGROUND

The pecan (*Carya illinoinensis* (Wangenh.) K. Koch) is one of the few native North American plant species that has developed into a significant agricultural crop (Wood et al. 1994).

An inherent characteristic of pecans is that they are alternate bearing, producing a higher-yielding crop followed by a lower-yielding crop. Alternate bearing has been, and continues to be, the cause of unstable prices and profits (McEachern 2013), due to the variation in quality. This phenomenon makes it difficult to estimate yield on a year-to-year basis, and it is known as one of the major problems affecting the entire supply chain from growers to marketers (McEachern 2019). It is also important to consider that even though the term biennial is associated with an "on" year followed by an "off" year, pecans can have more than two consecutive "on" or "off" years (Monselise & Goldschmidt 1982), making forecasting challenging.

Pecan tree crop forecasting is presented with obstacles not present in other horticultural crops. The pecan tree is extremely tall (12 to 16 m), making it difficult to perform nut counts. Furthermore, the rather inconspicuous aspect of the female flowers that blend in with the spring foliage (Wells 2017) make them hard to identify early in the season, thus hindering crop forecasting. Therefore, an early and accurate crop forecasting method would be extremely beneficial to the tree nut industry; informing the early determination of sales volumes and optimal management planning and budgeting.

Pecan yields depend on the number of pistillate "female" flowers, and the mechanisms that govern floral initiation are still poorly understood. To date, there are two main theories explaining variation in yields (Wood et al. 2004; Heerema 2006; Wells 2017): the Nutritional Theory and the Hormonal Theory. The Nutritional Theory is based on the influence of carbohydrates on yields, and the Hormonal Theory considers that the principal inhibitor of crops is hormones.

Carbohydrates (sucrose and starches) represent the nutritional building blocks of woody plants. Yield and vegetative growth are dependent on ready supply of carbohydrates and

how they are distributed between the sinks (Oliveira & Priestley 1988). Organs that produce more exporting carbohydrates like mature leaves are sources, while the non-photosynthetic organs are described as sinks (Hopkins & Huner 2008).

While extensive research has been conducted into carbohydrate movement in pecans (Martinez Diaz et al. 2012; Valenzuela-Núñez et al. 2019) and other crops (Hagidimitriou & Roper 1994; Nzima et al. 1997; McQueen et al. 2004; Bustan et al. 2011), the relationship between energy reserves and yield has not been established. The potential of carbohydrate sampling as a routine measurement for assessing the reserve energy status of the trees in an orchard, and hence the ability to predict and influence crops, has never been pursued in the Australian pecan industry.

Accurate yield estimation is essential in agriculture to provide management with timely information to improve decision making during the growing season and to improve supply chain management. This research focuses on understanding the effects of carbohydrates in crop phenology and the mechanism that drives yields. It also investigates mineral concentrations in different organs of the pecan tree and their relationships to yields. This knowledge is critical to improving the potential to set a heavier crop and develop an accurate yield forecasting tool. Currently, there is no scientific method to forecast yields before the crop has set in early spring (McEachern 2018), hence the importance of studying the potential for carbohydrates to provide early estimates. This dissertation provides the pecan industry with new information to work further in meeting the industry's need for reliable and precise forecasting tools using available resources.

1.2 THESIS AIM

Previous work has been limited to the study of the seasonal translocation of carbohydrates and their role in flowering. What is not yet known is how the carbohydrate pool impacts yields.

From the knowledge collected and discussed in the literature review chapter, it can be seen that carbohydrate status has the potential to be used as a management tool to assess tree

health. Therefore, the overarching aim of this research was to quantify carbohydrate and mineral concentration storage in pecans to better understand the carbon allocation and nutritional requirement patterns.

This research also involved the study of the dynamics of carbohydrates, nitrogen (N), phosphorous (P), and potassium (K) in pecan trees through different phenological stages, and how these are related to tree health and yields. The specific objectives of each chapter are as follows:

Chapter 3: The objective of the study described in this chapter was to monitor total non-structural carbohydrates (TNSC) in the trunk of pecan trees for five years to determine if subsequent yields could be predicted. For this study, 50 trees were selected from seven fields. Ten of the 50 trees were sampled monthly for the analysis of TNSC in the trunk. Yields were collected on a field basis each year. Environmental data from the on-farm weather station was also collected. The results provide an insight into seasonal carbohydrate movement and their relationship to yields.

Chapter 4: The objectives of the study described in this chapter were to monitor starch, soluble carbohydrates, nitrogen, phosphorous and potassium dynamics in the leaves, trunk, and lateral roots over 12 months to understand seasonal patterns within the different organs and how yields could impact storage and vice versa (ie., how resources determine subsequent yields). The results provided a needed insight into the seasonal fluctuations of carbohydrates and mineral within the trees.

Chapter 5: The objective of this chapter's experiment was to study the impact of pruning timing and severity on the carbohydrate pool. In this experiment, different pruning methods and timings were applied to the same field. Carbohydrate responses in the trunk, growth and estimated yields were monitored. It is important to mention that for this chapter, the plan was to harvest individual trees. However, due to the continual rainfall towards the end of the harvest and the subsequent risk of flooding, we did not have time to perform an individual tree harvest.

Chapter 6: This final chapter discusses the broader insights gained from the research, implications of the knowledge gained, and considerations for future research in the field.

CHAPTER 2 : LITERATURE REVIEW

2.1 *CARYA ILLINOINENSIS*

The pecan (*Carya illinoinensis* (Wangenh.) C. Koch) is a member of the *Carya* genus and is a nut native to North America. It was noted by Brison (1974) as the most important nut crop native to the United States. The pecan is classified as a hickory, belonging to the walnut family (*Juglandaceae*) (Wells 2017).

Pecans' native habitats are the floodplains of the Mississippi River, Ohio, Missouri, the Red Rivers and their tributaries, along many of the largest rivers of central Texas (Wood et al. 1994; Wells 2017), and isolated pockets throughout Mexico (Wood et al. 1994).

In 2017, 2018 and 2019, the countries most prominent in the pecan industry were Mexico and the USA producing an average of 49% and 43%, respectively; followed by South Africa at 6% (International Nut & Dried Fruit 2017, 2018, 2019). Pecans are also successfully grown commercially in other countries such as Australia, Israel, Argentina and Peru (Brison 1974; Rice 1994; Wells 2017). Australian pecan production represents only 1% of total world production (Figure 2.1).

According to Brison (1974), pecans were first grown in Australia at the Langbecker Nursery in Bundaberg (Queensland) in 1928, and the first commercial orchard was planted in Gympie. In 1963 Stahmann Farms Enterprises (SFE) became the first commercial producer and marketer in Australia, with the first farm located near Gatton. In 1971 it established its flagship farm in Moree, New South Wales (NSW) (Wilkinson 2005).

"Trawalla" the SFE farm near Moree, NSW (Figure 2.2) is currently the largest pecan operation in the Southern Hemisphere, encompassing over 1,000 hectares with 70,000 mature trees and 50,000 young trees (Hadgraft 2019).
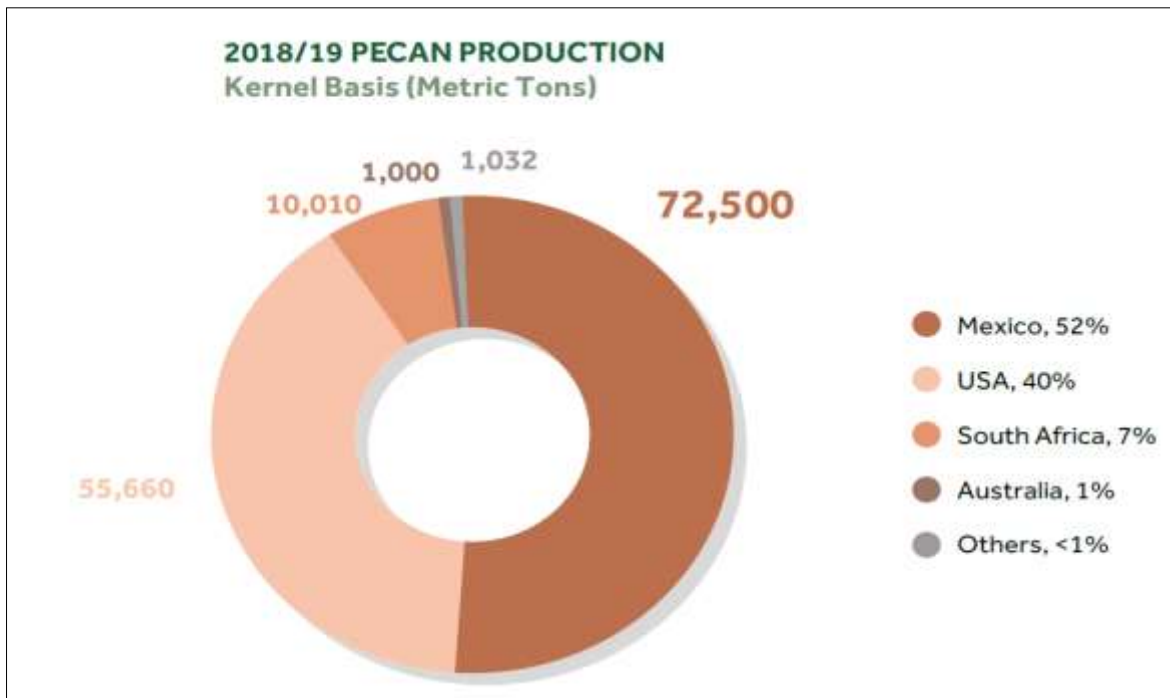
Figure 2.1: World pecan production (International Nut & Dried Fruit 2019)

Figure 2.2: Farm location in Australia (Google Earth 2015)

In the last few years, the value of Australian tree nut production at the farm gate has increased as a result of both increased demand and higher prices. Overall, the farm-gate value of Australian tree nuts is forecast to increase to $1.7 billion AUD by 2025 (Australian Nut Industry Council 2019). The global demand for tree nuts in general, and pecans in particular, continues to rise primarily due to increased focus on health and wellbeing (Simpsoms 2016). Several studies support the health benefits of tree nuts (Bao et al. 2013; Aune et al. 2016; Gulati & Misra 2017), while other studies have focused on the health benefits of consuming pecans (Rajaram et al. 2001; Haddad et al. 2006; McKay et al. 2018); making tree nuts an increasingly valuable crop.

2.2 PECAN PHYSIOLOGY

Pecan inflorescence are monoecious, both female (pistillate) (Figure 2.3) and male (catkin) (Figure 2.4) flowers are present on the same tree (Sparks 1992; Graham 2019), and dichogamous, meaning that at the time pollen is shed by the male flowers of a particular genotype, the female flowers of that genotype are not receptive to avoid self-pollination (Brison 1974; Sparks 1992; Graham 2019).

Wichita is a pecan protogynous cultivar with pistillate flowers first receptive when the protandrous cultivar, Western Schley, sheds its pollen. One to two weeks later, Wichita begins shedding pollen from its mature catkins when Western Schley's pistillate flowers become receptive (Figure 2.5).

Pecan fruit development evolves from pistillate flower anthesis and pollination to kernel filling and shuck dehiscence through the season. Nut development can be divided into two phases:

- Phase 1: From blossoming to shell hardening
- Phase 2: Filling and ripening period, shell hardening to shuck split (Worley 1994b; Byford 2005).

Maturity time varies among cultivars (Sparks 1992; Worley 1994b). Many cultivars take 180 days from the time of budbreak to maturity, with the earliest maturing pecan cultivar requiring only 137 days (Rice 1994).

In Australia, the period from flower to maturity occurs from September to April. Budbreak occurs in spring from late September to early October. Different cultivar buds break at different dates (Sparks 1992).

Pecans go through several stages of development; from pollination to full maturity. The phases and times of each period are described below in

Table 2.1 (Southern Hemisphere dates) and Figure 2.6 showing the development for the Northern Hemisphere.

Figure 2.3: Pecan floral structure. Cluster of four pistillate (female flowers) borne on terminal end of current season's shoot

Figure 2.4: Pecan floral structure. Staminate (male) flowers borne from lateral buds on previous season's shoot

Figure 2.5: Mature pecan tree "Western Schley", with adjacent rows of "Wichita" on the commercial far Trawalla

Table 2.1: Pecan development phase days for 'Shawnee' cultivar (adapted from Rice 1994)

Development Phase	Time (days)
Successful pollination	0
Post pollination and first nut drop; stigma in pistillate turns brown	6
Early nut expansion and fertilization; end of second nut drop	40
Rapid nut expansion; early water stage; third nut drop	60
Shell hardening begins	80
Shell hardening at the halfway stage; kernel filling begins	90
Kernel filling; early gel and dough stages; shell hardening complete	105
Dough stage; kernels are full size and near complete	130
Shuck split; nuts are fully developed	156

Figure 2.6: Development and maturation of pecans (Northern Hemisphere). Dates vary with season, location and variety (Wells 2007, as cited inBeuchat & Mann 2010).

2.3 ALTERNATE BEARING

Pecans are alternate-bearing, wind-pollinated trees. Throughout the years, scientists have agreed that the most significant problem facing the pecan industry is alternate bearing (Malstrom 1974; Wood 2012; McEachern 2019).

Alternate bearing is defined as the alternation of pistillate flowering (Wood 2011), subsequent nut yield (Wood 1990; Heerema 2006), and quality from one crop year to the next (Wood 1990). Pecans, in contrast with any other tree fruit that experiences alternate bearing, mature late in the season, leaving limited time before leaf fall to accumulate carbohydrates for the next season (Conner & Worley 2000). Trawalla exhibits biennial bearing patterns as shown below in (Figure 2.7).

Alternation or biennial production is present in many evergreen and deciduous fruit trees and has been studied widely (Monselise & Goldschmidt 1982). The fact that different types of trees such as avocados, pistachios, olives, mangos and apples show alternative bearing indicates that alternation is somehow inherent to the nature of the plant (Monselise & Goldschmidt 1982). Additionally, individual trees (Wood 1990), or even individual branches, can be out of sync with the rest of the tree (Monselise & Goldschmidt 1982; Wood 1990).

Scientists are divided between the two theories of irregular bearing (Wood et al. 2004; Wells 2017): Phytohormone Theory (PT) and Carbohydrate Theory(CT) (Wood et al. 2004). The latest research has demonstrated that a combination of both theories is relevant to explaining the irregular bearing problem (Liu et al. 1999; Wood et al. 2004; Wells 2017).

Flower initiation, specifically pistillate flowers, are the limiting factor in fruit production and, hence, the main cause for alternate bearing (Smith 2005; Thompson et al. 2019). Pistillate flowers are borne in the current season's wood (Crane et al. 1934). PT assumes that flowering is controlled by flowers suppressing or promoting phytohormones; ultimately influencing yields. Wood (2011) found that pistillate flower initiation is largely regulated by endogenous cytokinin-gibberellin ratio, with a partial modulation by auxin and ethylene.

CT assumes that the carbohydrate pool from the previous season controls returning blooms and crop size. Support for this theory has been provided by several scientists who found a positive relationship between carbohydrates and alternate bearing (Smith & Waugh 1938; Brison 1974; Wood 1995; Goldschmidt 1999; Wood 2014). Brison (1974) noted the importance of reserve storage from the previous season as a catalyst for a higher crop and, that a tree in an "off" year since it doesn't have a significant fruit load to nurture, stores the energy that will be used the following year. However, other studies have not been able to find a relationship between carbohydrates and alternate bearing. (Rohla et al. 2007) studied the effect of fruit thinning on the carbohydrate pool and organic bound nitrogen and potassium concentrations. This study concluded that thinning improved return bloom, but carbohydrates, nitrogen and potassium were not the limiting factors.

For this research, the influence of hormones in the alternating process is not ruled out. A large amount of literature (Wood 2011) shows that gibberellin and cytokine content is related to flowering. Also, Thompson et al. (2019) showed that exogenously applied plant growth regulators can affect pistillate return bloom, which suggests that they might also have some involvement in floral initiation. However, in this study, only CT was explored to determine the correlation between reserves and yields.

Figure 2.7: Trawalla yields in Tonne / ha from 2013 to 2021 for varieties Wichita and Western Schley

2.4 CARBOHYDRATES

Tree growth is driven by photosynthesis which is carried out in the presence of carbon dioxide, water, oxygen and sunlight absorbed by leaves. Photosynthesis's main function is to provide energy and carbon to support growth and ensure plant survival. Carbohydrates are the main product of photosynthesis, and are considered the main source of energy for trees. In trees, carbohydrates are stored primarily in the form of starch and soluble sugars (Chapin et al. 1990).

TNSC are the portion of a plant's soluble sugars (ie., sucrose, glucose, fructose and sorbitol) and a fraction of insoluble starches laid down in the plant structure (Oliveira & Priestley 1988). TNSC can be mobilized within the plant for metabolism or translocated to other organs. This translocation process is ruled by sink to source relations. Translocated carbohydrates move from the perennial parts to the growing tissues via the phloem (Oliveira & Priestley 1988). Organs that produce more assimilates exporting carbohydrates (like mature leaves) are sources; while the non-photosynthetic organs are described as sinks (Hopkins & Huner 2008).

In deciduous trees, like pecans, carbohydrates stored in the perennial organs are central to plant life and essential for maintaining tree function during dormancy and fuelling spring growth (Glerum 1980; Tromp 1983; Oliveira & Priestley 1988; Cheng & Robinson 2004; Da Silva et al. 2014).The carbohydrates pool is also important for maintaining hydraulic transport, particularly during episodes of severe stress (Sala et al. 2012), , but knowledge about the interaction between NSC reserves and xylem hydraulics is still very limited (Wang et al. 2018). Hence, the importance of the accumulation of carbohydrates during the growing season is critical to ensuring tree survival (Pallardy 2008; Regier et al. 2010).

As discussed previously, reserves are essential to support budburst in early spring after dormancy and play a crucial role in the ability of deciduous trees to tolerate freezing temperatures. Seasonal changes in carbohydrates in trees have been studied for over 100 years (Oliveira & Priestley 1988). There is an extensive literature on this topic (Oliveira & Priestley 1988; Wolstenholme & Whiley 1989; Loescher 1990; Herrera 1998). However,

there is no clear understanding of how the allocation of carbohydrates to storage reserves occurs in trees (Da Silva et al. 2014).

Pecan trees' early spring growth is primarily dependant on the carbohydrate reserves accumulated during the previous growing season. Smith and Waugh (1938) highlighted the exhaustive process of spring growth and nut filling that reduces carbohydrates considerably. Their findings are consistent with Brison (1974) who reported that when trees have a large crop, the available carbohydrates are required for healthy growth processes and the formation and maturity of nuts during the current season, thus compromising the food supply for the following spring.

Fruiting potential is associated with carbohydrate distribution between the vegetative and reproductive organs of the tree, or adequate source-sink relationships (Oliveira & Priestley 1988; Goldschmidt 1999; Hopkins & Huner 2008; Fisher 2009; Furze et al. 2018).

Smith and Waugh (1938) experiments measured the starch content of pecan roots. Samples of lateral roots with diameters ranging from 9.5 to 12.7 mm showed that starch content decreased in spring due to the growth and blossoming of the tree, and did not improve during summer or autumn because of the demand that a large crop puts on the tree.

In 2014, the macadamia industry investigated TNSC in macadamia trees and the relationship with light, climate, fruiting, growth and other agronomic factors (Vimpany 2016). From this research, it became clear that there is a high correlation between TNSC measured in the trunk and the phenological stage of the orchard, as shown in (Figure 2.8). The results showed that the tree accumulated reserves before flowering and those reserves started to decline until completion of the crop cycle. The carbohydrates seasonal fluctuations followed the same pattern for all varieties, with the A Series (Australian varieties) having a lower overall carbohydrate content than the H series (Hawaiian varieties).
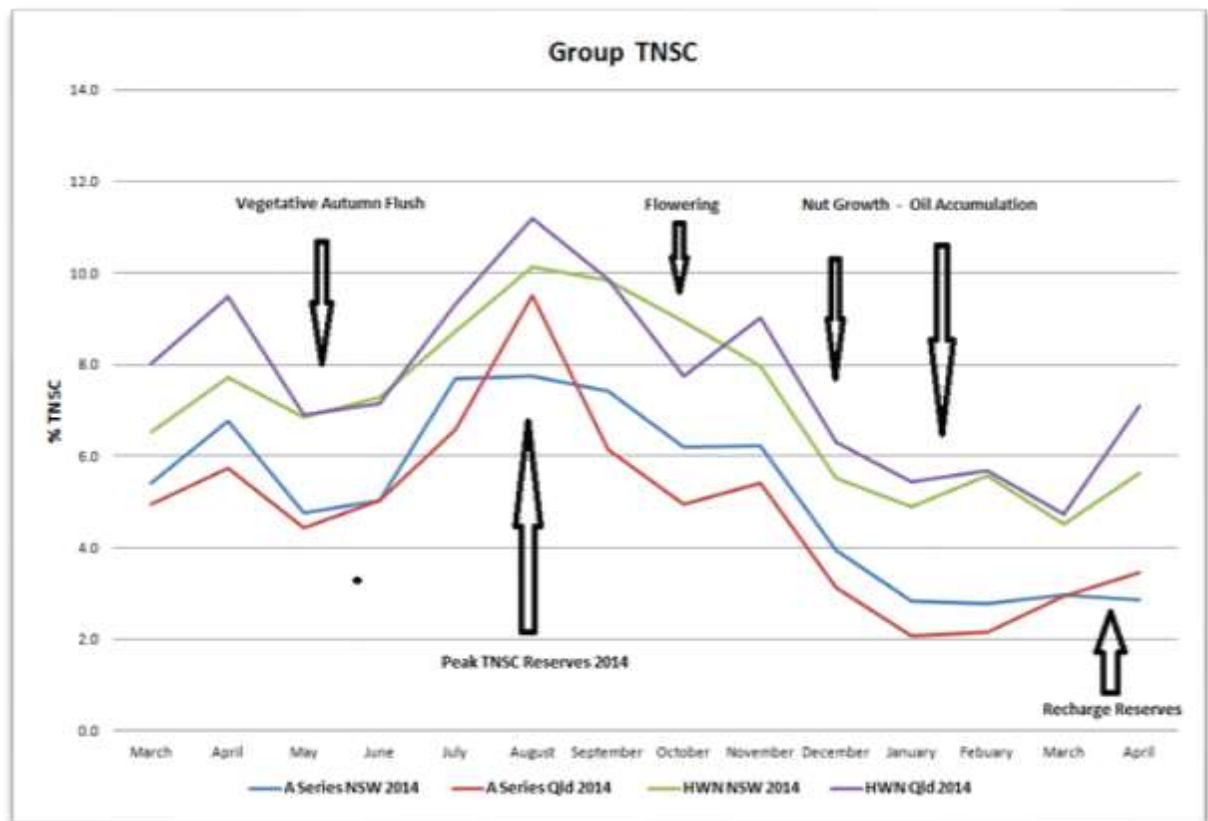
Figure 2.8: TNSC 2014 macadamia trial (Vimpany 2016)

2.4.1 Source-sink relationship

Carbon partitioning involves the transport of assimilates from source to sinks to ensure efficient distribution of energy and carbon between organs. Although a portion of the carbon produced is used by the leaf to support its growth and metabolism, the rest of the carbon is exported to the non-photosynthetic tissues in the plant via the phloem. It is estimated that up to 60% of produced carbohydrates are lost through respiration (Fisher 2009).

The direction of net assimilates to or from an organ is the determining factor in identifying sources and sinks. Sinks for photo assimilates comprise reproductive (flowers and fruits) and vegetative (shoots and roots) tissue. Plant organs can convert from a sink to a source (Kozlowski 1992). A good example of this conversion are the leaves in a deciduous tree. Initially, the tree will draw resources from other organs to develop in early spring,

functioning as a sink. However, once the leaves are fully formed, they will switch to being a source. TNSC are, therefore, accumulated and utilized towards carbon distribution and as a resource for reproductive and vegetative development (Hopkins & Huner 2008).

In trees, carbon is stored as carbohydrates, formed primarily by starch and soluble sugars (Chapin et al. 1990). In the leaves, sucrose is biosynthesised during photo assimilation and then becomes available for distribution from the leaves to the rest of the plant via the osmotic gradient of the phloem vascular system. Some photoassimilates are also stored in the leaves during the day as starch and, at night, this stored carbon is hydrolysed and further distributed within the plant to continue the mobilisation of leaf assimilates in the absence of photosynthesis (Smith & Stitt 2007).

Although the terms "source" and "sink" were originally applied to organs, they also can be applied at a cellular level (Kozlowski 1992). Carbon allocation is important as fruit production and quality depend on the adequate source to sink relationships. Hence, extensive research into carbohydrates has been performed on crops (Hopkins & Huner 2008).

It is important to note that carbon allocation is substantially different among the organs of the same plant, suggesting that carbon allocation is a programmed process that implies another measure of control beyond the source to sink relationship (Hopkins & Huner 2008; Sala et al. 2012).

2.4.2 Seasonal carbohydrates

The seasonal variations of carbohydrates have been studied extensively in numerous species (Cameron 1923; Smith & Waugh 1938; Whiley et al. 1996; Wolstenholme & Whiley 1997) for over 100 years (Oliveira & Priestley 1988).

There is a difference in annual patterns between deciduous and evergreen species. Deciduous trees' spring growth depends on stored carbohydrates while evergreen species, having leaves as a source for carbohydrates during dormancy, have a lower dependence

on carbohydrates to fuel re-growth (Wolstenholme & Whiley 1997). Further difference is found amongst species depending on their growth characteristics (Kozlowski 1992).

In pecan trees, a maximum concentration of carbohydrates in the trunk is observed in autumn after the crop has been harvested, followed by a decline in resources during winter. The decline in carbohydrates continues throughout spring and early summer until the leaves are fully developed. Once leaves are producing carbohydrates, resulting in a higher source input versus sink, carbohydrates will start to increase until they reach their peak in autumn. Smith and Waugh (1938) found a similar pattern for starches in the roots of pecan trees. Roots, however, will generally contain higher concentrations during winter when compared to other woody organs (Kozlowski 1992).

Management practices and environmental conditions affect carbohydrate concentrations. In autumn, the accumulation of sugars in vacuoles promotes the cold hardiness that protects trees from cold winter temperatures by decreasing the formation of intercellular ice (Kozlowski 1992). Hence, the importance of late-season accumulation of carbohydrates. When pecans reach fruiting, flower production and subsequent fruit set is a function of translocation during fruit development the previous year and the length of the period where leaves remain functional after fruiting (Davis & Sparks 1974). Early defoliation reduces the carbohydrate pool and reduces subsequent yields (Worley 1979b). Previous research (Smith et al. 1986) found that early fruit ripening varieties of pecans have a higher return bloom.

Early loss of pecan leaves affects the carbohydrate pool and prevents or greatly reduces yield the following year (Worley 1979a) . To increase carbohydrate production and storage for the following season, it is important to maintain healthy foliage after nut development. Adopting management practices that promote leaf retention will maximise carbohydrate production as leaf retention until frost is critical for minimizing alternate bearing (Sparks 1992). Other management practices have been proved to impact carbohydrate accumulation, eg., Oliveira and Priestley (1988) recommended the time of pruning to coincide with optimum carbohydrates to encourage a faster recovery.

2.5 TREE MANAGEMENT

Adequate growth and nut quality require sunlight, water and the availability of essential nutrients. Orchard management is critical to ensure optimal growing conditions. Deficiencies in any of these areas will impact tree health and crop potential.

2.5.1 Pecan nutrition and fertilizer

Pecan trees have significant macro and micronutrient requirements to ensure optimum development and crop quality. Although all the nutrients are essential for pecans, there are some macro and micronutrients that are critical for orchard productivity. The concentration of mineral nutrients in leaves influences their photosynthetic potential and reflects the nutrient conditions that influence tree health.

For most horticultural crops, fertiliser programs use foliar analysis to monitor the availability of nutrients in the soil during a period of the growing season. Leaf analysis is the standard method for determining the nutritional status of trees. Foliar analysis is the most accurate representation of a tree's nutritional status (Stafne et al. 2017). Standard concentrations of minerals in pecan leaves vary depending on the region. SFE leaf standards are given in Table 2.2. The standards are based on measurements taken in the month of February.

Table 2.2: Guidelines for leaf analysis at the Stahmann Farms Orchards, Moree, NSW, Australia for February (adapted from (Reibel 2019))

Element	Stahmann guidelines for leaf tissue
N	2.5 - 3.0%
P	0.12 - 0.3%
K	0.75 - 1.5%
Ca	0.7-2.5%
Mg	0.3-0.7%
S	0.15-0.25%
Na	<0.1%
Cl	<0.5%
Cu	5-50 mg kg^{-1}
Zn	50-100 mg kg^{-1}
Mn	150-500 mg kg^{-1}
Fe	50-300 mg kg^{-1}
B	20-50 mg kg^{-1}

2.5.1.1 Nitrogen

Nitrogen promotes leaf chlorophyll which increases photosynthetic potential (Tromp 1983) and delays leaf drop (Oliveira & Priestley 1988). Nitrogen can also affect flower initiation and cause abortion (Crane et al. 1934). Nitrogen stored in pecans trees is used preferentially in the spring followed by rapid nitrogen absorption (Acuña-Maldonado et al. 2003). A lack of nitrogen reserves has been proposed as a limiting factor after a large crop, and there is a considerable amount of literature supporting the role of nitrogen in tree health and yield (Kraimer et al. 2001; Wood & Reilly 2001; Cheng & Robinson 2004; Kraimer et al. 2004). Other literature, however, has found no evidence of a relationship between nitrogen and yield in pistachio trees (Crane & Al-Shalan 1997).

2.5.1.2 Potassium

Potassium is an activator for many enzymes essential for photosynthesis, carbohydrates, and protein synthesis. Potassium affects osmotic potential; a principal factor in plant movement through the phloem (Hopkins & Huner 2008), playing an important role in transporting carbohydrates. Wood et al. (2010) studied the effect that elevating potassium had in the crop, it concluded that by improving tree potassium through soil banding of potash increased in shell nut yield, increased nut quality and reduced fruit drop. Since non-structural carbohydrates and organically bound nitrogen is transported to the phloem (Vreugdenhil 1985), potassium is critical for fruit development and yield. However, the link between NSC and N and K as relevant to fruit development and yield is not clear.

2.5.1.3 Phosphorous

Phosphorous is readily mobilized and distributed within plants (Hopkins & Huner 2008). It plays an important role in the energy metabolism of cells, making it essential for cell division and the development of the growing tip of the plant (NSW Department of Primary Industries n.d). In pecans, phosphorous is an important element for energy storage and is fundamental to the production of wood and fruit (Wells & Conner 2007) reduces leaf scorch prevents early defoliation and is an important nutrient in nut growth (Rohla 2013). Deficiencies in Phosphorous results in limited branching, incomplete separation of nuts from husk at maturity and slightly delayed defoliation (Brison 1974).

2.5.2 Irrigation

Irrigation is applied in pecan orchards to ensure adequate water supply to support tree growth and nut production. The quality of nuts produced depends on an adequate water supply. Irrigation can dramatically improve the quality of the kernel (Worley 1994a).

Water demand is driven by crop load and maturity. The demand increases as the season progresses, showing a peak demand during kernel fill (Wells 2021).

The average rainfall in Moree is 612 mm per annum, with approximately 400 mm precipitation over the months of the pecan season (Bureau of Meteorology 2021). The water required to achieve optimal soil moisture levels when the rainfall is not enough, is applied by irrigation.

The main methods applied in the pecan orchards are flooding and drip irrigation. Stahmann Farms' orchard (Trawalla) was initially set up as a flood irrigated orchard, but gradually parts of the farm have converted to drip irrigation to improve water efficiency (Figure 2.9).
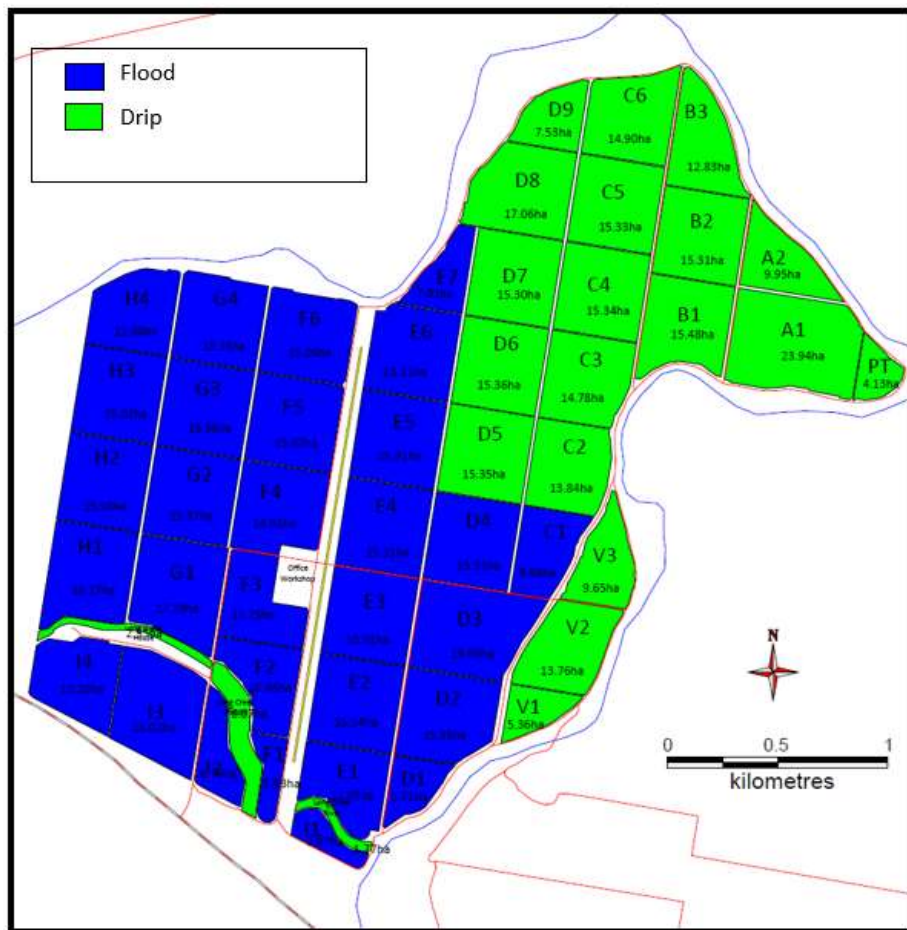
Figure 2.9: Map of different irrigation types in Trawalla Pecan Orchard, Moree, NSW, Australia

2.5.3 Pruning

The main objectives of pruning are to improve light interception by allowing more light penetration into the canopy, stimulating growth that will produce fruiting limbs in the following season (Brison 1974). Pecan trees are naturally forest trees that must compete for light. They exhibit apical dominance; meaning they put much of their energy towards the tips that are receiving the most light. This ensures that they can quickly dominate the light within a forest and thus out-compete their neighbours. In nature, the trees will shade out their lower limbs which will eventually be lost (Sparks 2005). So, it makes sense to prune a productive well-managed orchard periodically to improve light interception as pecan yields appear to be limited by shading. Also, hedge pruning and topping are recommended to improve spray coverage. Pruning is an important management practice for various reasons. Previous studies have confirmed the benefits of pruning (Heerema et al. 2016; Wells 2018).

2.6 YIELD FORECASTING

Accurate nut yield estimates in the field before harvest are critically important for production management decisions and marketing (Weckler et al. 2015). Pecan trees present major obstacles when assessing yields. The trees are extremely tall (Wood 1970; Brison 1974). In Australia, 10 to 12 metres in height is ideal. The other obstacle when forecasting yields is the rather inconspicuous aspect of the female flowers which blend in with the spring foliage (Wells 2017), making them hard to identify early in the season. Carbohydrates have been identified as a potential marker for predicting yields (Wolstenholme & Whiley 1989; Barwick 2011), however other studies have identified nitrogen as the element responsible for both vegetative and fruiting growth in bearing apples (Cheng & Robinson 2004).

Pecan yield forecasting literature is limited and, unfortunately, the majority of forecasting models investigated are accurate only when the crop has already been set (Wood 1970; Wright et al. 1990). Sparks (1996, 1997) developed a set of equations for yield prediction that achieved an R^2 of 0.908 for arid conditions and R^2 0.945 for humid conditions. Sparks

(1997) developed a complex of eight variables, five of them directly or indirectly related to rainfall during critical tree and fruit developmental stages in the current and previous season. The limitations with this model are: 1. Depending on the area of plantation, it will have different parameters and 2. It was based on yields for a region and cultivar rather than a farm-field level.

2.7 SUMMARY

This review examined the factors that affect pecan yields, with an emphasis on the phenomenon known as alternate bearing. To better understand the mechanisms responsible for pecan yields, it was necessary to first discuss pecan physiology. The pistillate flowers or their absence is what will determine the potential yield for the season. Initial discussions regarding the formation of pistillate flowers focused on the role of either carbohydrates or phytohormones as the cause of alternation in pistillate flowers. However, the latest literature available suggests a combination of carbohydrates and phytohormones as the reason for the variation of high yield "on" years followed by "off" years. From this review, it is clear that pecan yields are also affected by a number of management factors, including light interception, water and nutrition.

From the knowledge collected and discussed in this chapter, it can be seen that though carbohydrates are not the only factor affecting pecan tree bearing. Carbohydrates have the potential to be used as a management tool to assess tree health and predict subsequent yields. Carbohydrate sampling and testing is an inexpensive exercise. Phytohormone quantification, on the other hand, is still challenging due to low levels in the plant and their chemical diversity. Previous work has been limited to studying the seasonal movement of carbohydrates and their role in flowering. No information about the use of carbohydrates as a management tool was found in our review of the literature.

Therefore, the overarching aim of this research was to quantify the carbohydrate and mineral concentration reserve status in pecans to better understand the carbon allocation and nutritional requirement patterns, and how these are related to yields.

CHAPTER 3 : STUDY OF TOTAL NON-STRUCTURAL CARBOHYDRATES IN PECAN (*CARYA ILLINOINENSIS* (WAGENH)), AND ITS RELATIONSHIP TO YIELDS.

3.1 ABSTRACT

Carbohydrates are considered essential for maintaining tree function during dormancy and supporting early spring growth. Deciduous trees draw in stored reserves to fuel budburst and support shoot growth until the canopy has enough leaves to become autotrophic.

To characterise seasonal patterns of TNSC in pecan varieties Wichita and Western Schley and to study their relationships to yields, the amount of TNSC in trunks and annual production were recorded on a monthly basis for five years (2015-2020) in seven fields. Climate data was also collected from the onsite weather station for the period of the experiment. Annual production was determined for the fields and varieties studied in this chapter.

Seasonal TNSC behaviour was similar for the varieties studied however, the quantity differed significantly between them, suggesting that variety affects the ability to harvest carbohydrates. TNSC in the trunk of both species had a significant moderate correlation with the environmental parameters of temperature, solar radiation and rainfall.

TNSC was greater early in the season for "on" years and generally lower in the summer. A dramatic decrease in the concentration of TNSC (%) was observed for the varieties studied in June (early dormancy) preceding an "on" year.

Moderate positive correlations R = 0.52, P<0.01 for Wester Schley and R= 0.63, P< 0.05 for Wichita, were observed between averaged TNSC for the months September and October of the current season, coinciding with late spring, early summer and June of the current season, coinciding with the early dormancy with subsequent yields. This is an important

finding that suggests the potential use of carbohydrates concentration in the trunk in winter and spring to predict yield.

3.2 INTRODUCTION

The pecan (*Carya illinoinensis* (Wangenh.) K. Koch) is one of the few native North American plant species that has developed into a significant agricultural crop (Wood et al. 1994).

An inherent characteristic of pecans is that they are alternate bearing, producing a higher-yielding crop followed by a lower-yielding crop. Alternate bearing, which has been the cause of unstable prices and profits (McEachern 2013), results from the failure of pecan trees to initiate a sufficient number of pistillate flowers (Amling & Amling 1984). This phenomenon makes it difficult to estimate yield on a year-to-year basis, and is known as one of the significant problems affecting the entire supply chain; from growers to marketers (McEachern 2019). It is also essential to consider that, as well the biennial characteristic (an "on" year followed by an "off" year), pecans can have more than two consecutive "on "or "off" years (Monselise & Goldschmidt 1982), making crop forecasting challenging. An early and accurate crop forecast would be extremely beneficial in the tree nut industry to determine sales volumes and optimum management planning and budgeting.

There are two main theories explaining alternate bearing (Wood et al. 2004; Heerema 2006; Wells 2017). The Nutritional Theory, which is based on the influence of carbohydrates in yields, and the Hormonal Theory where the principal inhibitor of crops is hormones.

Carbohydrates (sucrose and starches) represent the nutritional building blocks of woody plants. Carbon assimilation and translocation are critical for plant survival and resistance to disease (Sala et al. 2012). Yield and vegetative growth are dependent on the level of carbohydrates and how they are distributed between the sinks (Oliveira & Priestley 1988). Organs that produce more assimilates like mature leaves are sources, while the non-photosynthetic organs are described as sinks (Hopkins & Huner 2008).

While extensive research has been conducted into carbohydrate movement in pecans (Martinez Diaz et al. 2012; Valenzuela-Núñez et al. 2019) and other crops (Hagidimitriou & Roper 1994; Nzima et al. 1997; Iglesias et al. 2003; McQueen et al. 2004; Bustan et al. 2011), a relationship between energy reserves and yield for this crop has not been established. Carbohydrate sampling has the potential to offer a routine measurement to assess the reserve energy status of the trees in an orchard, and hence provide the ability to predict and influence crops as has never been possible in the pecan industry. Furthermore, little work has been done on seasonal changes in carbohydrates segregated by "on" and "off" years.

The analysis of TNSC seasonal patterns could provide an opportunity for growers to assess whether their trees are healthy and performing well. Thus, the overarching aim of this study was to analyze the TNSC and their impact on tree productivity. Tracing shifts in the TNSC pool is vital to studying plant responses to environmental and management changes. Furthermore, understanding how TNSC change throughout the season and its relationship to yields has practical implications for managing an orchard to improve carbohydrate reserves and tree health, and ultimately yields.

This study was conducted to determine the seasonal patterns of TNSC in the trunks of pecan trees and the potential to use these patterns as a yield forecasting tool.

3.3 MATERIALS AND METHODS

3.3.1 Site details

This research was performed at the commercial SFE farm near Moree, NSW, Australia, in the property "Trawalla." Trawalla consists of 735 ha, with a total of 69,000 50-year-old trees planted in a 10m by 10m grid spacing. The farm is divided into fields, with each field made up of several irrigation blocks (Figure 3.1).

The main pecan cultivars grown at Trawalla are the varieties Western Schley (synonym Western) and Wichita (Figure 3.2), and they are planted in alternate rows. Every second row is planted with the pollinator variety Western Schley. These cultivars were chosen for

their pollination characteristics as cross-pollination is necessary for the optimum crop set (Reibel 2019). The variety Wichita female flower is receptive before shedding pollen (Type II Cultivar). In contrast, the variety Western Schley sheds pollen before the female flower is receptive (Type I Cultivar), hence the suitability of these two cultivars.

Figure 3.1: Map of Trawalla farm, located in Moree, NSW, Australia showing block layout (Google Earth, 2021)

Figure 3.2: Kernels of varieties Wichita (left) and Western Schley (right), 1 cm grid paper

3.3.2 Experiment layout

For this study, 50 trees in two rows in the middle of the field were selected from seven fields randomly selected. In two of the fields, only variety Wichita trees were chosen. For the rest of the fields, variety Western Schley trees were selected, as shown in Figure 3.3. These fields were selected in order to have fields covering the two types of irrigation (flood and drip). Fifty uniform trees were selected in each field in two to three rows. The trees and rows selected are detailed in Appendix I: Tree sampling layout. One in every five trees, as shown in

Table 3.1, was sampled monthly for the analysis of TNSC in the trunk. An example of the sampling method for January 2014 (trees ending in 1-5) means that trees with numbers 1, 5, 11, 15, 21, 25, 31, 35, 41 and 45 were sampled. Samples were taken from August 2014 to July 2020.
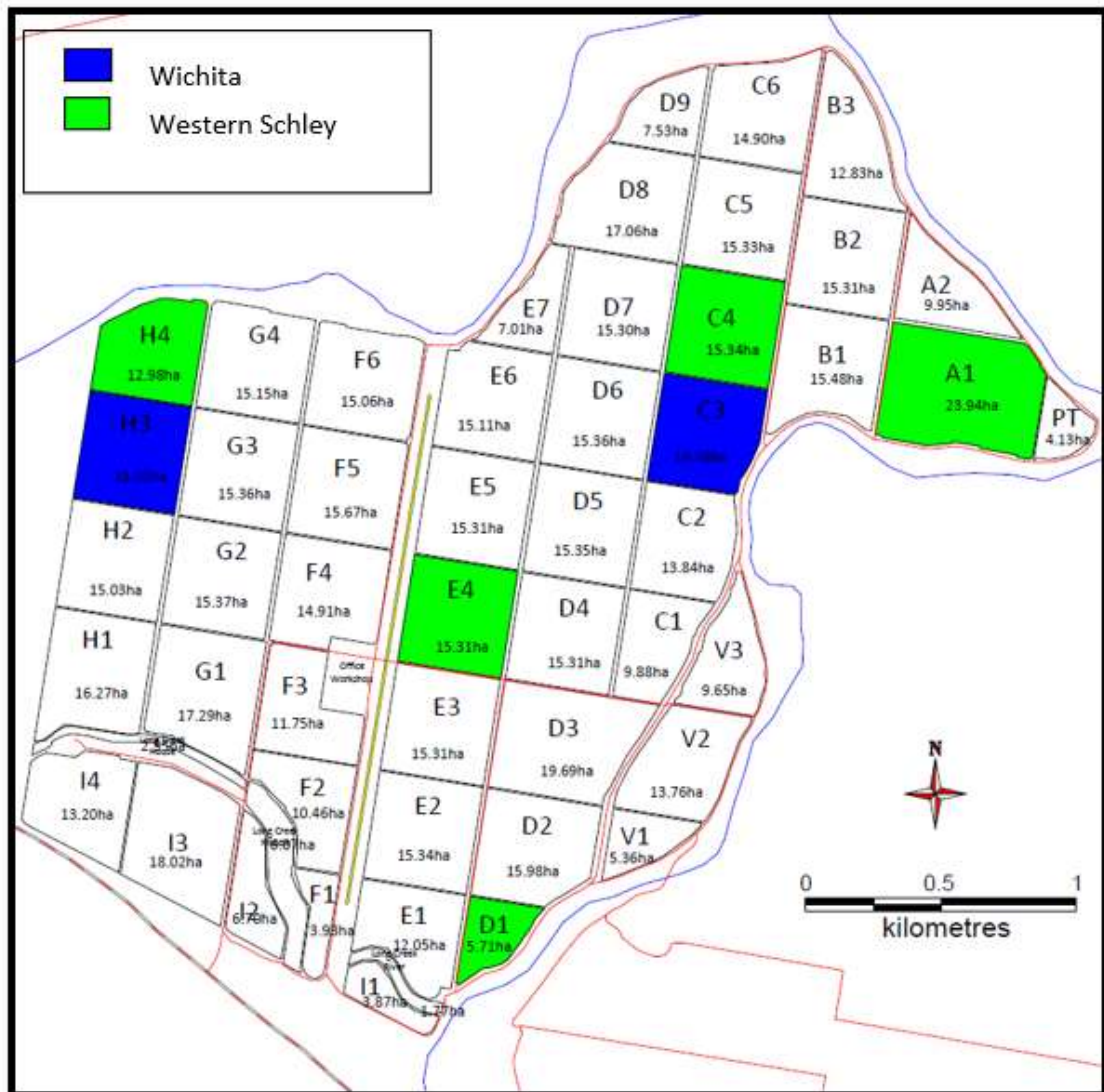
Figure 3.3: Trawalla map indicating fields and varieties chosen for the experiment

Table 3.1: Final digit of trees sampled each month out of the 50 trees

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2014	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6
2015	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8
2016	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5
2017	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7
2018	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9
2019	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6	3, 7	4, 8	5, 9	1, 5	2, 6

3.3.3 Carbohydrate sampling technique

Sample collection took place in the trunk of the tree, 1.5 m above the ground using a 25 mm spade drill bit, following the method described by Stephenson et al. (1989) and Vimpany (2016). We drilled until the sapwood was reached, and a sample of 4 mm depth was taken from that point (Figure 3.4). A set of callipers was used to measure the depth. The samples were then placed in a dehydrator for 24 hours at 70° C. After drying, the samples were ground using a Foss ^{TM} Cyclotec ^{TM} and sent to the New South Wales Department of Primary Industries (DPI) for testing. All field samples were collected on the same day. Sample collection and report example are provided in

Appendix II: Laboratory forms.

Figure 3.4: Sample collection, bark is being collected using a 25mm spade drill bit. Sample is collected in a foil tray

3.3.3.1 Determination of TNSC

The determination of TNSC was performed by the New South Wales Department of Primary Industries (DPI) as described in detail below by Meyer (2020).

The first stage of the starch analysis involved extracting soluble sugars with 80% ethanol at 80 °C, centrifuging the mixture, and removing the supernatant. This step was repeated, and the resulting pellet of soluble sugars was then resuspended for starch digestion.

When analysing TNSC, resident sugars are not extracted from the samples but carried through the starch digestion procedure, and quantified in the analytical finish.

Starch hydrolysis proceeded in two phases. In Phase I, the aim was to convert starch to soluble fragments with minimal production of glucose from the starch. In Phase II, the starch dextrin was quantitatively hydrolysed to glucose by amyloglucosidase.

Thermostable α-amylase hydrolyses starch into soluble branched and unbranched maltodextrins (1).

(1) Starch + H2O $\rightarrow$ maltodextrins

α-amylase (Spezyme FRED, Enzyme Solutions), pH 7.0 or 5.0, 80 °C

Amyloglucosidase (AMG, MEGAZYME) quantitatively hydrolyses maltodextrins to

D-glucose (2).

(2) Maltodextrins $\rightarrow$ D-glucose **Amyloglucosidase**

Flow Injection Analyser analytical finish

The extracts were preserved with 0.2% benzoic acid and made up accurately to volume. The filtered extracts were analysed by a continuous flow autoanalyzer using the alkaline ferricyanide decolouration method. This method involves the hydrolyzation of the starch and sugar extracts to invert sugar by 1 mol/L hydrochloric acid and heating (90 °C). The extracted invert sugars are dialysed into an alkaline stream of potassium ferricyanide and heated (90 °C). Invert sugar reduces yellow ferricyanide to colourless ferrocyanide.

The decrease in colour at 420 nm is directly proportional to the invert sugar (measured as glucose) present (Meyer 2020).

3.3.4 Environmental parameters

The following parameters were sourced from the Measurement Engineering Australia (MEA) farm weather station: air temperature, solar radiation and rainfall.

Figure 3.5: MEA weather station located at Trawalla airstrip measuring air temperature, solar radiation and rainfall.

3.3.5 Data analysis

Pearson correlation and multiple regression analyses were performed for the data obtained in the experiment. The distribution and temporal variation of TNSC were tested using ANOVA. Statistical analysis was carried out using IBM SPSS Statistics (version 22). Differences were considered significant at $P<0.05$.

3.3.5.1 Outliers identified in the data set

The results presented below are averages for the five years of data collected in order to find outliers. Figure 3.6 shows the yearly pattern of TNSC for Western Schley, with Figure 3.7 showing the yearly pattern for Wichita. Results were checked against the laboratory reports to see if the outliers were due to transcript errors. After ensuring that the outliers were not due to transcript errors, we decided to continue to use the outliers throughout the analysis as the aim of this research was to find correlations to yields, so it is important that we consider all the data points available. The outliers identified are shown in Table 3.2.
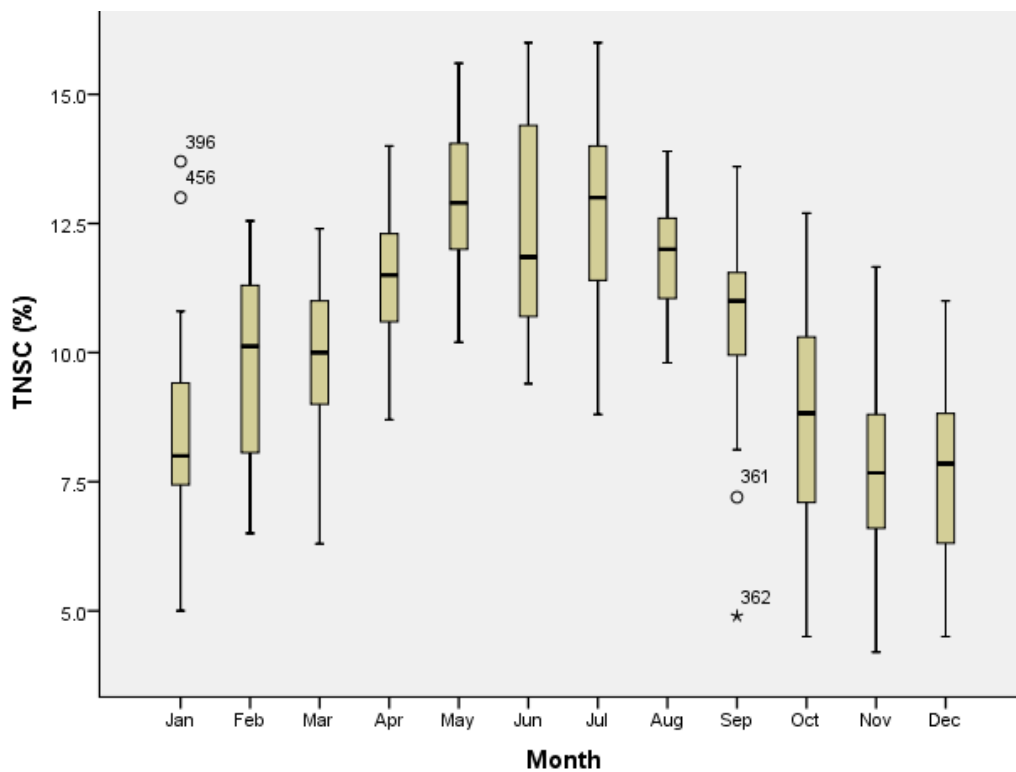
Figure 3.6: Boxplot monthly TNSC (%) for variety Western Schley from 2014 to 2020
N=293
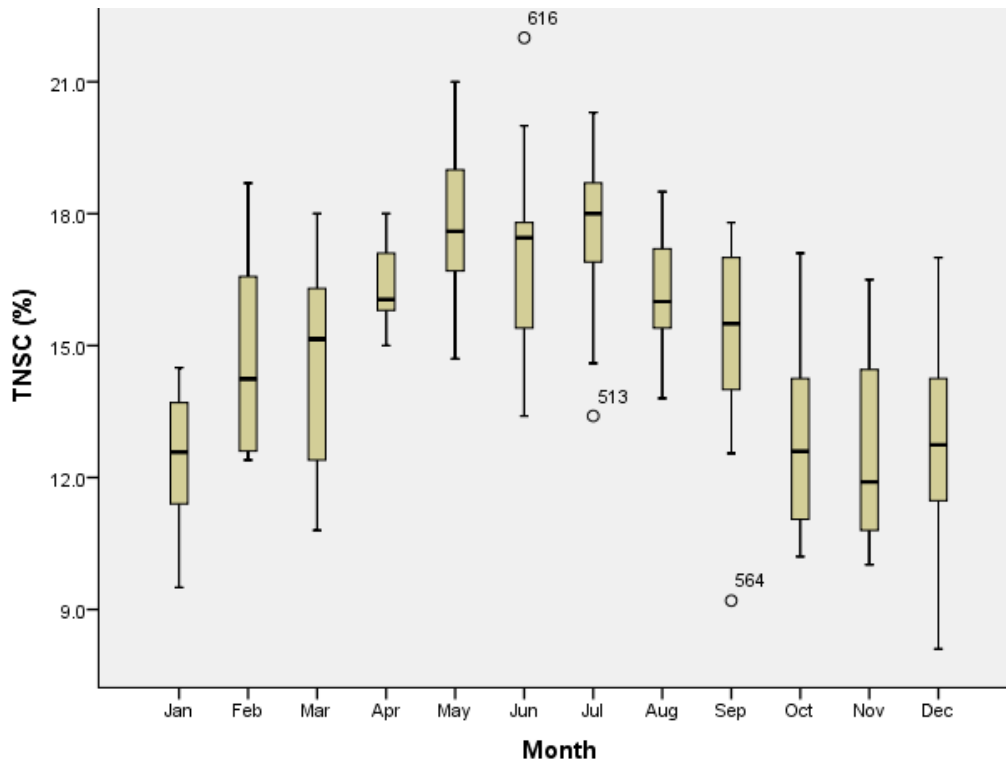
Figure 3.7: Boxplot monthly TNSC (%) for variety Wichita from 2014 to 2020 N=196

Table 3.2: Outlier values identified

Case	Field	Year	Month	Value
396	A1	2019	Jan	13.7
456	A1	2020	Jan	13
616	H3	2020	Jun	22
513	H3	2015	Jul	13.4
564	C2	2017	Sep	9.2
361	A1	2017	Sep	7.2
362	H4	2017	Sep	4.9

3.4 RESULTS

3.4.1 Seasonal changes in TNSC

The amount of TNSC in the trunk of both cultivars changed seasonally. The seasonal patterns of TNSC in the trunk appeared consistent for both varieties assessed in this study (Figure 3.8 and Figure 3.10). The only apparent difference was the higher content of carbohydrates (%) in the variety Wichita, which was a significant difference to the variety Western Schley at $P<0.01$ (Appendix III: Supplementary results Table 0.1).

For both varieties, a significantly higher concentration of TNSC was observed in autumn and winter compared to summer and spring (Figure 3.10).

There was a significant decrease in TNSC in the spring before budbreak. This shift in resources resulted from the use of carbohydrates by the perennial tree for bud development. After budbreak, TNSC levels remained low until early summer during nut sizing. Levels were then restored as the trees began using carbohydrates produced by their leaves. Energy demand was less than the energy provided by photosynthesis. The accumulation of TNSC in the trunk slowed temporarily during February during nut sizing. These results are consistent with the high energy required at the time of nut sizing. After nut sizing, TNSC levels in the trunk continued to increase steadily until June when there was a drop that coincided with the start of dormancy.
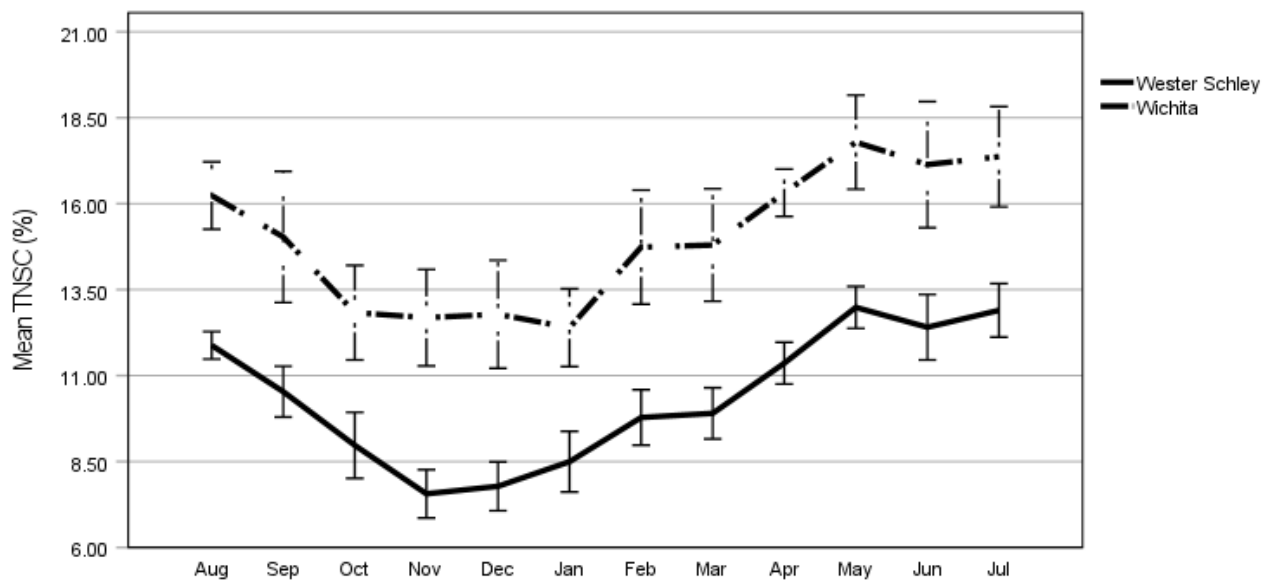
Figure 3.8: Monthly seasonal changes in TNSC (%) in varieties Wichita (dashed line) and Western Schley (solid line). Averaged values from data collected between 2014 to 2020
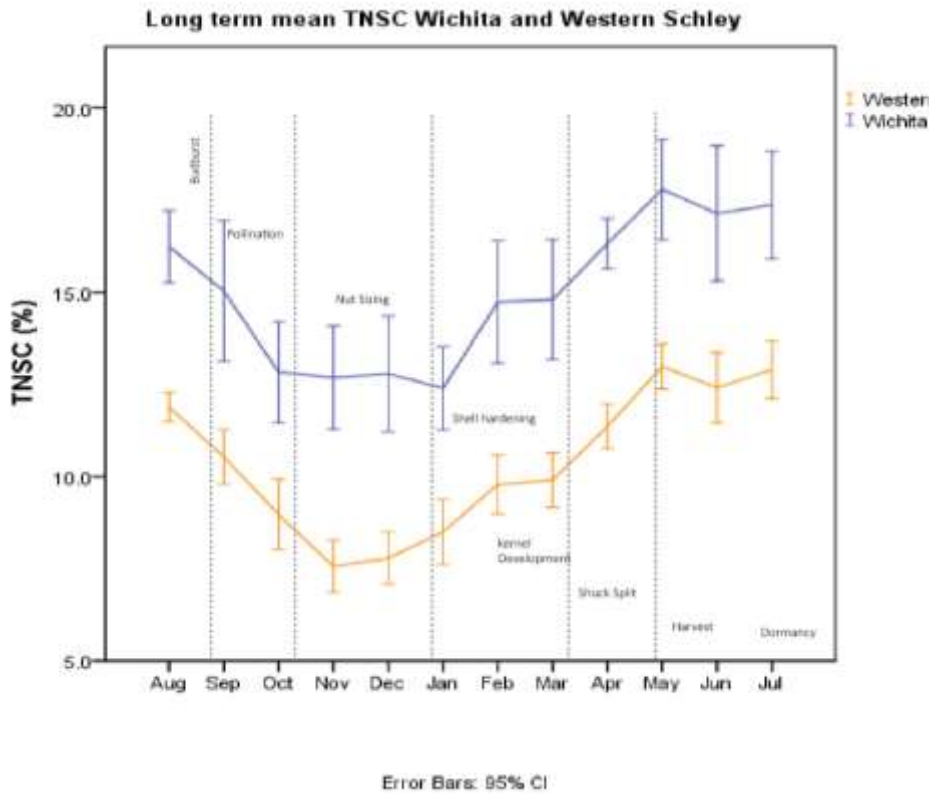
Figure 3.9: Seasonal changes in TNSC in varieties Wichita and Western Schley
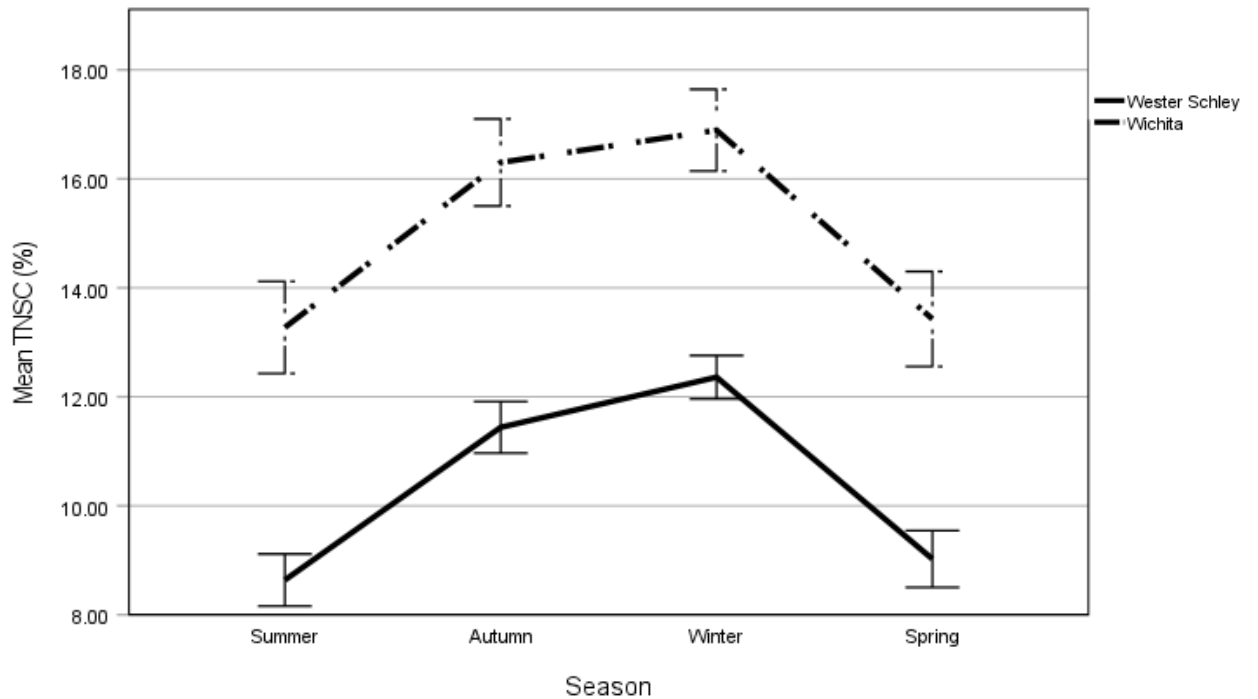
Figure 3.10: Average TNSC in varieties Wichita (dashed line) and Western Schley (solid line) by season

3.4.2 Seasonal changes in TNSC for "on" and "off" years

The TNSC (%) values obtained from August 2014 to July 2020 was segregated by years with higher than average yields "on" years and years with yields lower than the long-term average, "off" years on a field basis. The number of fields in each category are represented below (Table 3.3 and Table 3.4). Yields higher than the average yields, 2015 to2021, for the same field and variety were considered "on"year. Values lower than the average were considered "off" year.

Variation in the concentration of TNSC was found when segregating monthly values during the crop season, leading to "on" years versus "off" years values. Both varieties had a higher concentration of TNSC in the trunk during October, going into an "on" year coinciding with the start of pollination. Supplementary results are provided in Appendix III: Supplementary results, Table 0.2. A dramatic decrease in the concentration of TNSC (%) was observed in June preceding an "on" year. After pollination, both varieties experienced an increase in carbohydrates. This increase was more pronounced during the years preceding an "off" crop. Additional analysis is available in Appendix III: Supplementary results, Table 0.2.

Table 3.3: Count of number of fields showing "on" or "off" status per season for both Wichita and Western Schley varieties

Crop year	Period sampled	Fields "on"	Fields "off"
2014-15	Aug 2014- Mar 2015	6	1
2015-16	April 2015- July 2016	2	5
2016-17	April 2016- July 2017	6	1
2017-18 *	April 2017- July 2018	0	5
2018-19	April 2018- July 2019	7	0
2019-20	April 2019- July 2020	1	6

* Fields C3 and E1 were not sampled during this period.

Table 3.4: Field yields in Kg.ha^{-1} from 2014 to 2020, on years are represented in bold

Yields	A1	C2	C3	D1	E4	H3	H4

2015	**3,169.9**	**3,715.0**	2,407.0	**5,930.5**	**4,487.1**	**3,800.0**	**3,750.8**
2016	2,410.8	2,342.5	**4,323.9**	1,356.8	1,811.4	**2,281.7**	3,554.2
2017	**3,287.6**	**3,762.7**	2,613.0	**3,549.8**	**3,429.5**	**3,779.9**	**3,629.5**
2018	1,262.4	1,980.1		985.7		993.8	1,113.4
2019	**4,209.6**	**3,725.1**	3,581.2	4,898.4	3,971.5	**3,387.1**	3,129.4
2020	510.5	1,119.2	1,839.6	1,298.1	1,104.6	2,575.1	3,661.6
Average (2015-20)	**2,475.1**	**2,774.1**	**2,952.9**	**3,003.2**	**2,960.8**	**2,802.9**	**3,139.8**
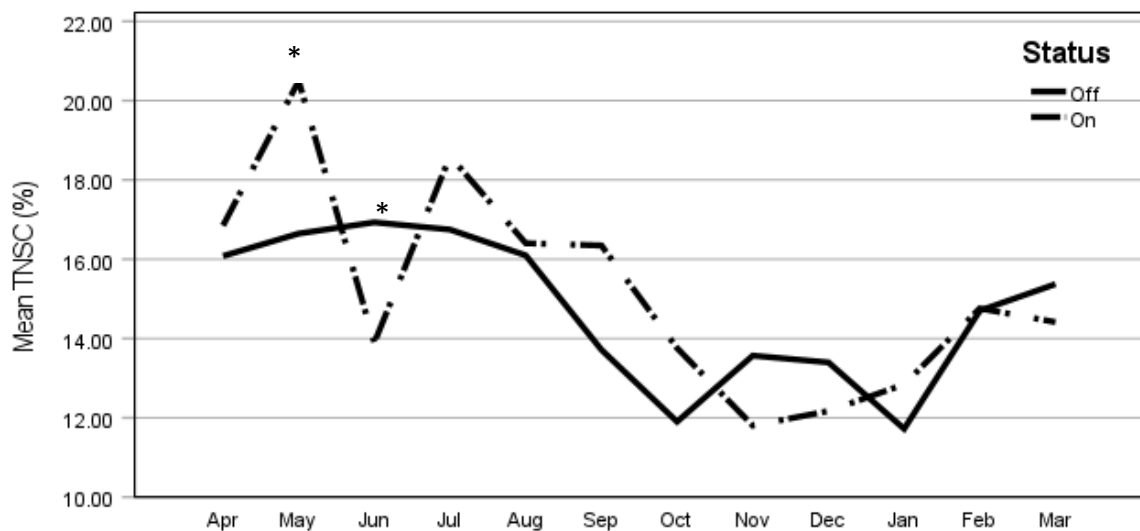
Figure 3.11: TNSC (%) in the trunk from April to March for "on" and "off" years for variety Wichita trees (* indicates a significant difference at the 0.05 level). Dashed line showing average values for "on" fields from 2014 to 2020 and solid line "off" fields from 2014 to 2020
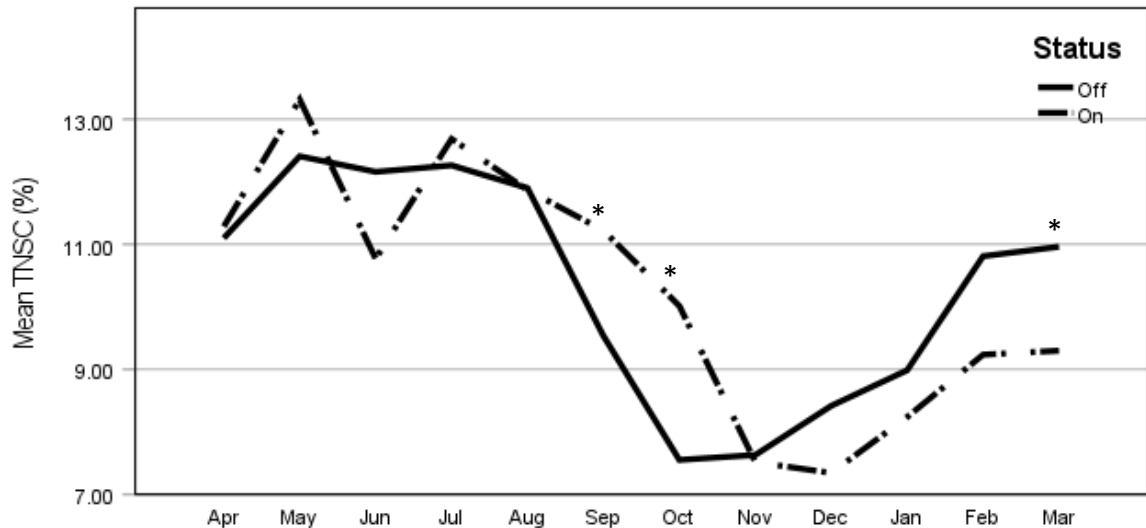
Figure 3.12: TNSC (%) in the trunk from April to March for "on" and "off" years for variety Western Schley trees (* indicates a significant difference at the 0.05 level). Dashed line showing average values for "on" fields from 2014 to 2020 and solid line "off" fields from 2014 to 2020

3.4.3 Correlation between TNSC (%) and field yields by crop year

Australian pecan crops are harvested from late April to July. For this experiment, the crop year was defined as April to March, for example April 2018 to March 2019 affecting the 2019 crop harvested in May-June.

Correlation analysis was performed on the data set by variety and month. A significant correlation to the level $P<0.05$ was found for both varieties in June.

Regression analysis indicated that 66% of variety Wichita's and 33% of variety Western Schley's variation in subsequent season yields (kg/ha) was negatively associated with TNSC (%) in the trunk in June (winter).

No significant correlation was found between the parameters described for the other months in the Wichita variety. However, other correlations were established for the

months of September, October and March for Western Schley (Table 3.5). The variety Wichita was only sampled in two fields, whereas the variety Western Schley was sampled in five fields. So, the final number of cases for the Wichita analysis was smaller than the number of cases obtained for the variety Western Schley.

Analysis of the average values of carbohydrates in September and October returned a significant positive correlation for both varieties, as shown in Table 3.6. This suggests that higher average carbohydrates at the time of budburst is associated with higher yields.

Table 3.5: Coefficients of correlation of TNSC in the trunk in the months preceding harvest to yields in varieties Wichita and Western Schley. Correlations between TNSC (%) and yields from fields monitored from 2014 to 2020

	Correlations	
	Western Schley	Wichita
Apr	-.035	.516
May	.225	.707
Jun	**-.574***	**-.810***
Jul	-.064	.145
Aug	-.085	-.046
Sep	**.539****	.549
Oct	**.406***	.445
Nov	-.163	-.538
Dec	-.336	-.248
Jan	-.206	.369
Feb	-.499*	-.049
Mar	**-.698****	-.372

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 3.6: Coefficients of correlation of TNSC in the trunk averaged for the months of September and October to yields in varieties Wichita and Western Schley

	Correlations	
	Western Schley	Wichita
September-October	.518[**]	.626[*]

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

3.4.4 Correlation between TNSC (%) and field yields for the preceding year

The Australian pecan crop is harvested from late April to July. For this experiment, we assessed the relationship between carbohydrates in the preceding year, from October to March. Below, in Table 3.7, we can see an example of the months assessed October 2014 to March 2015 affecting the 2016 crop.

Correlation analysis was performed on the data set by variety and month. A significant correlation to the level $P<0.01$ was found for both varieties in October.

Regression analysis indicated that 61% of variety Western Schley's and 60% of variety Wichita's variation in yields (kg/ha) were negatively associated with TNSC (%) in the trunk in October Figure 3.13

No significant correlation was found between the parameters described for the other months (Table 3.8).

Table 3.7: Example crop year and previous crop year summer months for 2016 yields. Represents the month and year sampled affecting in this example the 2016 crop

Month	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Year		2014						2015									2016				
Event							Harvest 2015												Harvest 2016		
Previous crop year TNSC (%)							x	x	x	x	x	x	x	x	x	x	x	x			
Previous crop year summer TNSC (%)	x	x	x	x	x	x															

Table 3.8: Coefficients of correlation of TNSC in the trunk to yields in varieties Wichita and Western Schley

	Western Schley	Wichita
Oct	-.781**	-.774**
Nov	-.388	.136
Dec	.117	-.160
Jan	-.277	-.441
Feb	-.068	-.367
Mar	.032	-.394

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)
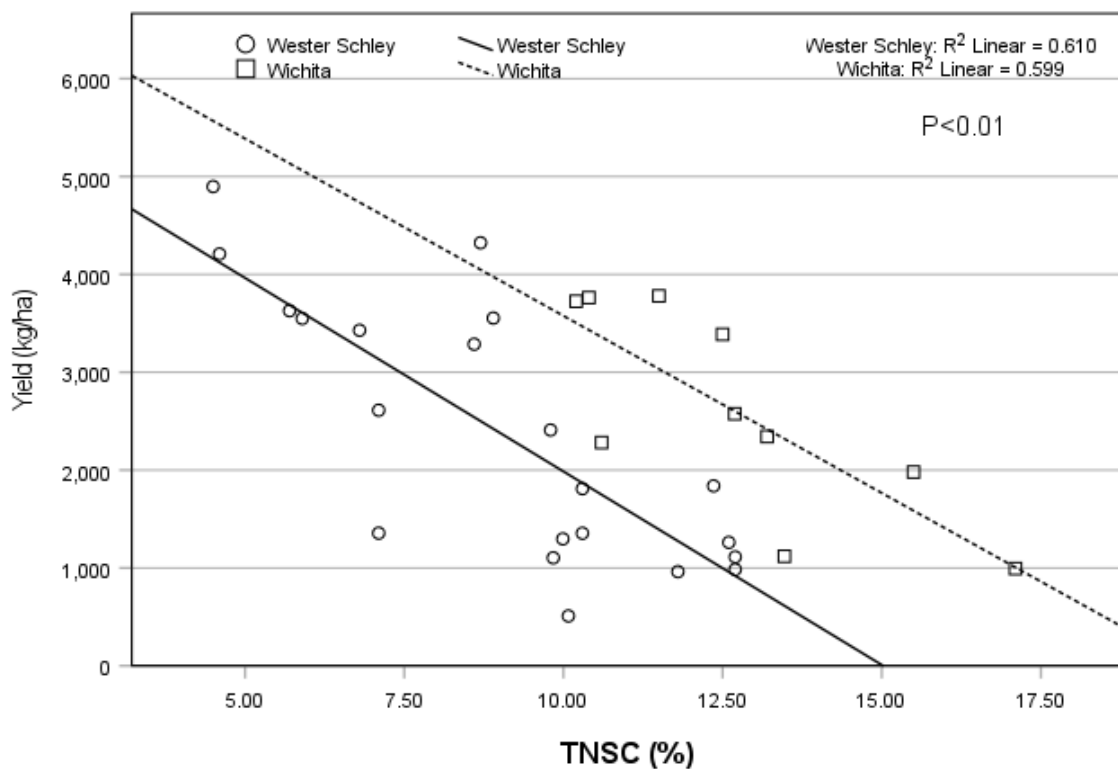
Figure 3.13: Linear regression of the relationship of field yields (kg/ha) by TNSC (%) in the month of October previous season for varieties Wichita and Western Schley

3.4.5 Influence of environmental parameters on TNSC

Average monthly rainfall, temperature and irradiation obtained using the on-farm weather station were compared to the monthly average TNSC from September 2015 to March 2020.

3.4.5.1 Correlation analysis for TNSC in the trunk and temperature

The value of the correlation between TNSC in the trunk and temperature had an R^2 of 0.49 for variety Western Schley and 0.36 for variety Wichita. This correlation was significant at the level P<0.01 (Figure 3.14). There was no violation of the normality and independence assumptions. This result means that 49% of variety Wichita's and 36% of variety Western Schley's of the variation in the concentration of TNSC can be explained by temperature.
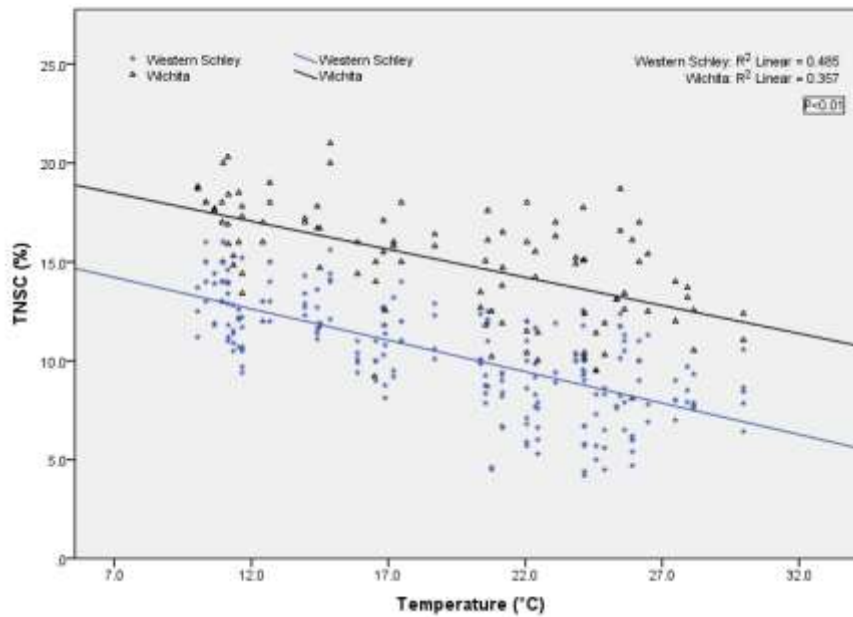
Figure 3.14: Relationship between TNSC and average temperatures for varieties Wichita and Western Schley

3.4.5.2 Correlation analysis for TNSC in the trunk and solar radiation

A significant moderate correlation was found between TNSC in the trunk and solar radiation. The R^2 value for variety Western Schley was 0.52 and 0.41 for variety Wichita
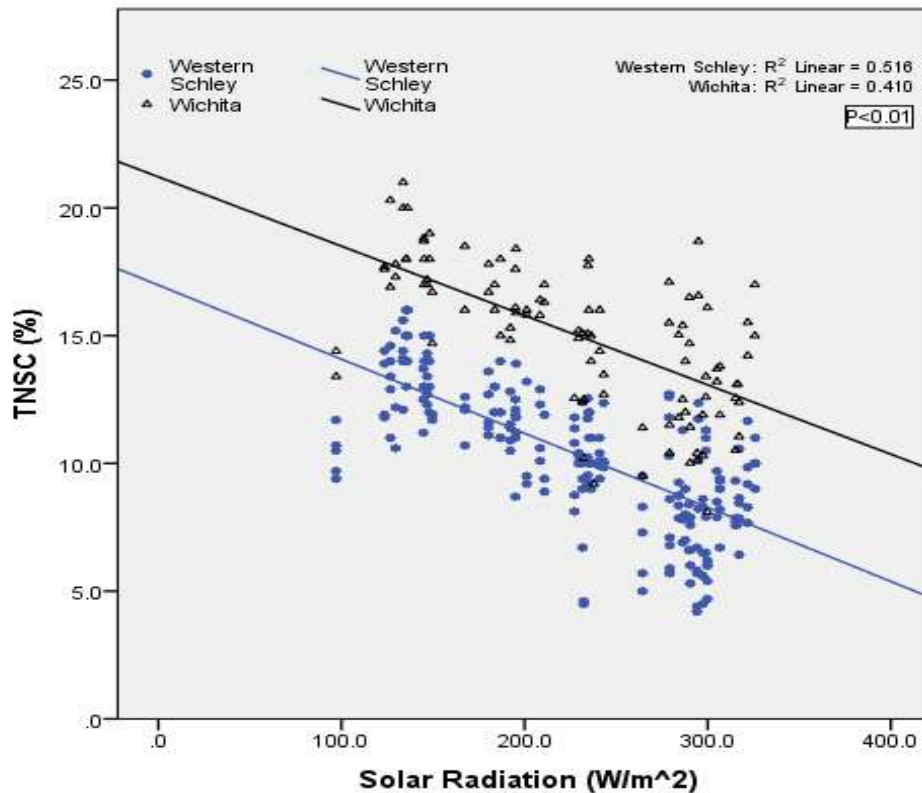
Figure 3.15: Relationship between TNSC and solar radiation for varieties Wichita and Western Schley

3.4.5.3 Correlation analysis for TNSC in the trunk and rainfall

No correlation was found between TNSC in the trunk with rainfall.

3.4.5.4 Multiple correlations and regression analysis for TNSC in the trunk

The values of multiple correlation R^2 between TNSC in the trunk and the environmental parameters (temperature, solar radiation and rainfall) were 0.55 for variety Western Schley and 0.421 for variety Wichita. Correlation is significant at the level P<0.01. There was no violation of the normality and independence assumptions. This result means that 55% and 42% for varieties Wichita and Western Schley, respectively, of the variation in the concentration of TNSC, can be explained by the combination of temperature, solar radiation and rainfall.

The regression equation obtained was:

Variety Western Schley

Predicted TNSC = 17.387 – 0.11 (Rainfall) – 0.96 (Temperature) -0.021 (Solar radiation)

Variety Wichita

Predicted TNSC = 21.431 – 0.006 (Rainfall) – 0.055 (Temperature) -0.022 (Solar radiation)
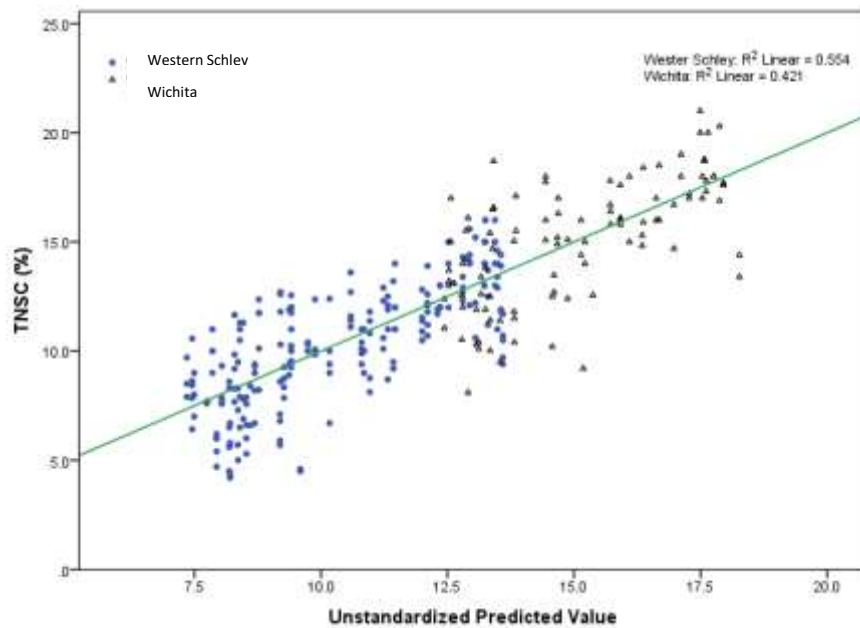
Figure 3.16: Relationship between TNSC and unstandardized predicted values from multiple correlations for the varieties Wichita and Western Schley

3.5 DISCUSSION

The seasonal patterns for both varieties considered in this research were similar to those previously reported in other studies (Smith & Waugh 1938; Krezdorn 1955; Worley 1979a). In this studies starch concentrations tended to reach maximums in the late fall and minimum concentration in early summer. In our research, total carbohydrate concentration in the trunk steadily decreased from spring to early summer, and subsequently increased to a peak in autumn, then slowly reduced during winter. A minimum was achieved in June, coinciding with the start of the Australian winter and pecan

dormancy. The winter minimum is believed to be caused by the conversion of starch to sugars during the hardening process (Worley 1979a).

While there were similarities in the carbohydrates' seasonal patterns for the varieties studied, there were significant differences in TNSC quantities. These differences could be related to the capacity of different varieties to store carbohydrates and their respective dates for budburst and defoliation (Wood et al. 2003). Generally, variety Wichita's budburst occurs several days before variety Western Schley's, and it holds its foliage for a longer period when compared to variety Western Schley.

The seasonal patterns in both varieties differed when segregated by year going on to an "on" and "off" crop year. TNSC was higher in the "on" year fields from dormancy up to October, supporting the hypothesis that carbohydrate concentration is greater early in the season for "on" years (Wood & McMeans 1981; Nzima et al. 1997; Wood 2014). TNSC were generally higher in the summer in "off" trees. Similar results have been obtained in studies performed with pistachios (Crane & Al-Shalan 1997). A minimum value of TNSC was obtained for fields going onto an "on" year before dormancy. These results contradict the results of studies in olive trees where "on" versus "off" carbohydrates showed no difference in patterns in carbohydrate concentrations (Bustan et al. 2011).

A significant correlation was found for both varieties in September and October (budburst-flowering) and the month of June (early dormancy) with subsequent yields. We also found that the TNSC concentration of the previous year (October) was negatively correlated to the yields. These results partially support the general Carbohydrate Theory, which predicts that fruit set is proportional to dormant season carbohydrates (Nzima et al. 1997; Wood et al. 2003) and is consistent with the results obtained by Worley (1979b) who reported a strong linear correlation between November's (May-June in the Southern Hemisphere) non-structural carbohydrates and subsequent yields.

The present study indicates that there is a moderate relationship between carbohydrates and subsequent crop. However, it is essential to note that these findings do not necessarily disagree with the role that phytohormones play in crop sets. Additionally, it is important to consider the stresses that a crop is under from prediction to harvest date. Hence, it is

reasonable to hypothesise that fruit set, rather than the final crop load, is dependent on the carbohydrate pool.

Our study also shows that the accumulation of carbohydrates in the trunk is influenced by temperature, solar radiation and rainfall. A significant moderate regression was obtained for the negative relationship between TNSC and temperature. Variety Wichita was found to accumulate a greater amount of carbohydrates than variety Western Schley. These results are similar to those found by Briceño Contreras et al. (2019) for the relationship between starches in the roots of both varieties and temperature. This result suggests the role of environmental cues in carbohydrate allocation throughout the season.

There is an opportunity for the pecan industry to continue research in this field to build a comprehensive yield forecasting tool using carbohydrate status combined with environmental parameters. Furthermore, we believe that the next step is to study how management practices on-farm could affect the available carbohydrate pool. Such as different nutrition and water inputs.

CHAPTER 4 : STORAGE DYNAMICS IN THE MINERAL ELEMENTS AND CARBOHYDRATE CONTENT OF THE LEAVES, TRUNK AND LATERAL ROOTS IN PECAN TREES (*CARYA ILLINOINENSIS* (WAGENH))

4.1 ABSTRACT

Pecan yields vary substantially from one year to the next due to a number of factors: climatic events, water stress, fertilization and the alternate bearing nature of the tree. Alternate bearing refers to the tendency of the pecan trees to follow a low production year with a higher production year. In pecan trees (*Carya illinoinensis* (Wagenh)), carbohydrates and minerals are considered essential for tree resilience during dormancy and critical to supporting early spring leaf growth before photosynthesis and significant mineral root uptake occurs.

A monthly survey of TNSC, soluble carbohydrates, macronutrients nitrogen, phosphorous, potassium and the micronutrient zinc (Zn) in different tree organs (leaves, trunk and exposed lateral roots) was conducted for a year. Annual production was determined for the surveyed trees in 2019, 2020 and 2021.

The objective of this study was to determine the seasonal changes in the mineral elements and carbohydrate content of the leaves, trunk and lateral roots, and how these affected fruiting potential. Alternate bearing was evident in some of the studied trees. The Alternate Bearing Index (I) in the individual trees assessed in this study ranged from 0.17 for the tree with the least alternation to 0.97 for the trees showing a stronger alternation pattern.

Starch was the primary storage carbohydrate. Both starches and soluble sugars declined from September to December, immediately after the budburst. As the season progressed, an increase was observed for both starches and soluble sugars except for the decline in sugars observed during kernel fill.

Best fit regression analysis indicated no association between potassium or phosphorous in the roots or trunk. There was a moderate association between TNSC in the trunk and early

spring, and this was related to subsequent yields. Nitrogen content during the same period was negatively correlated to the previous crop and positively correlated to subsequent yields. Nitrogen content in the trunk was positively correlated to the previous yield and negatively correlated to subsequent yields during November, coinciding with the nut set.

A significant correlation was found in leaf TNSC, nitrogen and phosphorous in November 2019 (during nut set) and yields harvested in 2020. These data do not confirm or reject the role of sugars and nutrients in the crop however, they provide a significant predictive tool for assessing potential crop size early in the year. Further work to quantify the contribution of storage carbohydrate and nutritional status to yields is justified.

4.2 INTRODUCTION

In pecan trees, adequate growth and optimum nut quality require sunlight, water and the availability of essential nutrients. Sunlight is critical to provide energy and carbon to support growth and ensure plant survival. Photosynthesis is the process by which plants use sunlight combined with water and carbon to transform light energy into chemical energy in the form of carbohydrates.

Perennial tree crop survival and yield potential are directly correlated to the carbohydrates synthesized during the season and how these are distributed between organs (Oliveira & Priestley 1988). Early spring growth, yield efficiency and productivity are largely influenced by the reserve's storage and availability during dormancy.

Nutrition is one of the management practices that has the potential to make a difference in production and is critical for tree survival and production. For instance, a lack of nitrogen reserves has been proposed as a limiting factor after a large crop, and there is a considerable amount of literature supporting the role of nitrogen in tree health and yields (Kraimer et al. 2001; Wood & Reilly 2001; Cheng & Robinson 2004; Kraimer et al. 2004).

Carbohydrates and nutrition are of paramount importance in pecan growth. Understanding how carbohydrates and other elements are distributed throughout the tree and how this changes during the season has important implications for managing nutrient

and carbohydrate reserves to improve tree health and yields. Hence, this research aimed to establish and quantify the seasonal dynamics of carbohydrates and the nutrients nitrogen, phosphorus and potassium between the different organs studied, with an emphasis on fruiting potential and as a forecasting tool.

The information on nutrients and carbohydrates could be useful for management decisions. For instance, the demands and requirements of the crop at different stages could be used as an indicator for fertilizer quantity and application timing.

4.3 MATERIALS AND METHODS

4.3.1 Site details

This research was also performed at the commercial SFE farm, Trawalla. For this study, 10 variety Wichita trees with similar trunk cross-sectional areas (TCSA) from different fields were selected randomly from the mature trees(Figure 4.1). Each tree was considered a experimental unit. The trunk diameter at 1.5 m above soil level was 14.13 cm (STDV 0.08), measured once at the beginning of the study.

Samples were taken monthly in three organs of the selected trees: leaves (from November to April), trunk and exposed lateral roots (above ground). Each sample was analysed for TNSC, soluble sugars, nitrogen, phosphorous and potassium.

The research was carried out for 12 months from October 2019 to September 2020. Yields from the selected individual trees were obtained by mechanically shaking each tree and manually collecting the nuts for the 2019, 2020 and 2021 harvests (Figure 4.2).
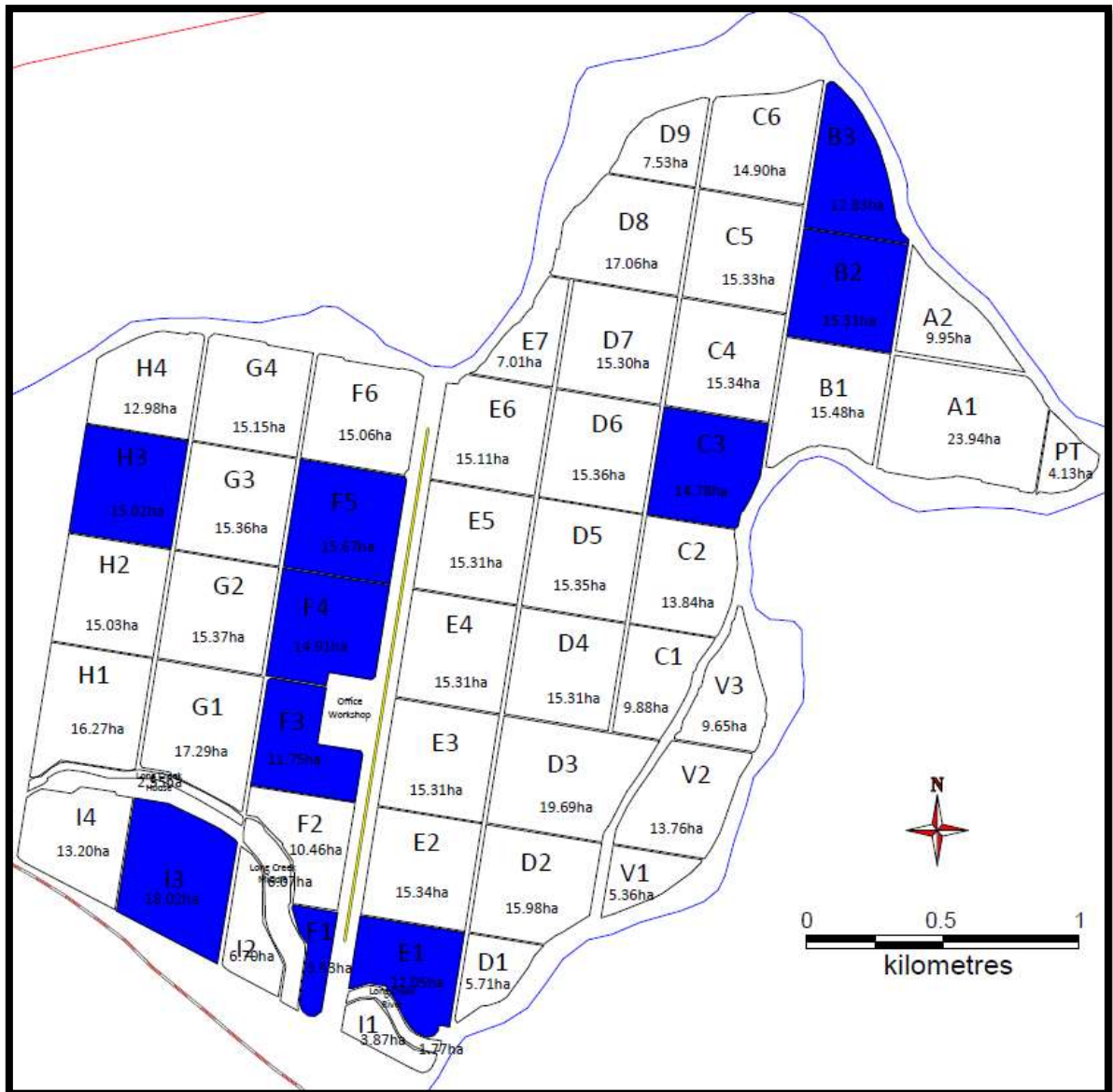
Figure 4.1: Trawalla farm showing fields where individual Wichita trees were selected (in blue)

Sampling	2019									2020												2021				
	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May
NSC, N, P,K , Zn Leaf																										
NSC, N, P,K , Zn Trunk and root																										
Yield																										

Figure 4.2: Sampling schedule for Wichita trees selected

4.3.2 Leaf sampling

Leaves were taken from the middle of the shoots of medium length and, when possible, from shoots bearing nuts. The leaves were washed with tap water using a colander, rinsed in distilled water, and dried at 70 °C for 24 hours. The dried samples were then sent to the New South Wales DPI laboratory for testing.

4.3.3 Determination of TNSC and soluble sugars

The protocol for the determination of TNSC was described in Section 3.3.3.1. Determination of starches were obtained by subtracting soluble sugar content from the value obtained for TNSC. Soluble carbohydrates were determined using Alkaline Ferricyanide method (AFIA Laboratory methods manual 2005).

Sample collection took place in the trunk of the tree and in the exposed lateral roots as shown below (Figure 4.3).
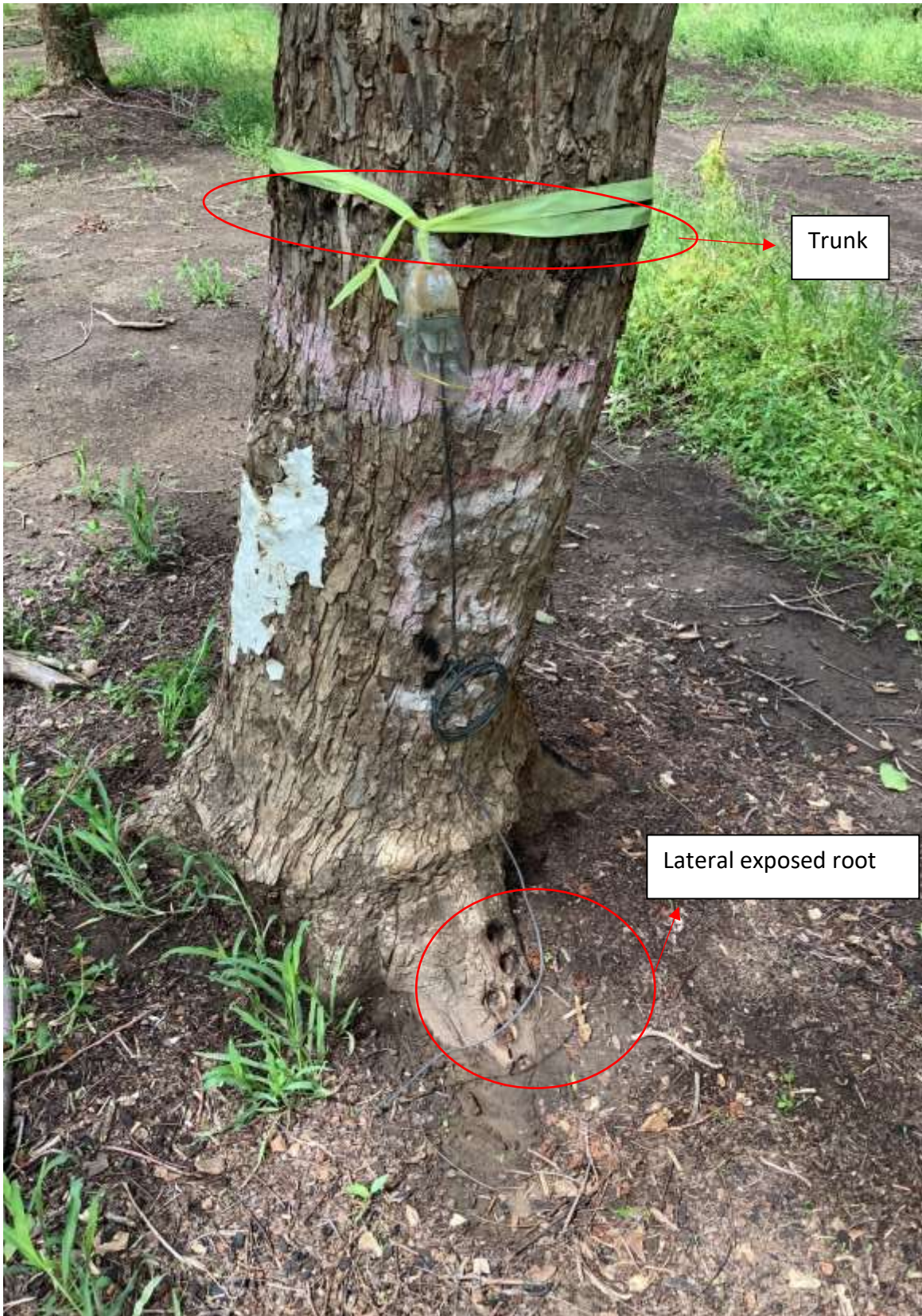
Figure 4.3: Monitored tree showing sampling area

4.3.4 Phenological tree status

During the season, phenological status was monitored and noted. Budbreak, fruit abscission and leaf abscission events were noted to represent phenological changes in the tree. Budbreak was recorded on the date when at least 50% of the buds showed green growth, as shown in Figure 4.4. The fruit abscission event was selected as the day the harvest started, and leaf abscission when the second harvest finished (at least 80% of leaf drop).

Figure 4.4: Budbreak example in pecan tree at the Trawalla farm

4.3.5 Harvest

The experimental trees were shaken using a commercial Orchard-rite® shaker and the nuts fell onto a large sheet of mesh cloth. The tree was shaken until 100% of the nuts fell from the tree. The harvested nuts were handpicked and weighed, as shown below in Figure 4.5.

A composite sample was taken from each tree for quality analysis. A hand crack out (HCO) was performed for a 453 gm sample. Each sample was weighed and cracked by hand. The kernel was then retrieved and weighed in order to obtain kernel recover.

The percentage of kernel recovery was calculated using the following formula:

% Kernel recovery = Weight of kernel/ initial nut in shell (NIS) sample x 100

I is the severity of alternate bearing, which is equal to:

$$I = \frac{1}{n-1} * \sum \frac{\left| a_{i+1-a_i} \right|}{a_{i+1+a_i}}$$

Where n is the number of years and a_i is the yield for the corresponding years.

I ranges from 0 to 1. When I is 1, the alternate bearing is 100%, and when I is 0, there is no alternate bearing (Pearce & Dobersek-Urbanc 1967).

Figure 4.5: Nuts being hand collected from one of the research trees

4.3.6 Data analysis

Pearson correlation and multiple regression analyses were performed for the data obtained in the experiment. Using each tree as a replicate and each month of sampling as treatment, the data obtained for carbohydrate and mineral concentration was analysed by the Analysis of Variance, ANOVA.

Statistical analysis was carried out using IBM SPSS Statistics (version 22). Differences were considered significant at $P<0.05$. LSD Post hoc was selected for the analysis of this set of data. Significant Pearson correlations were reported when the number of samples was equal to or greater than 7 as in some months it was not possible to collect the samples due to environmental challenges such as rainfall or the fields being flood irrigated at the time of sampling.

4.4 RESULTS

4.4.1 Yields

The yield data presented in Table 4.1 shows that the studied trees exhibited a biennial pattern, with an average I of 0.26. 2021 was an "on" year with yields averaging 33.5 kg/tree. 2020 was an "off" year for the majority of the trees assessed, averaging 13.6 kg/tree. 2021 had the highest yields of the three years monitored with an average of 39.85 kg/tree.

Table 4.1: Tree diameter, yield and alternate bearing intensity for variety Wichita pecan trees

Tree	Diameter (cm)	Yield 2019 (kg)	Yield 2020 (kg)	Yield 2021 (kg)	Alternate Bearing Intensity (I)
1	40.3	33.55	6.15	40.65	0.71
2	44.7	21.50	4.85	47.85	0.72
3	44.1	28.65	23.75	39.30	0.17
4	48.2	39.30	0.05	38.30	1.00
5	46.6	25.10	31.30	8.85	0.33
6	44.9	43.25	0.60	45.20	0.97
7	44.1	36.50	18.25	41.10	0.36
8	41.4	-	23.85	42.95	-
9	48.1	-	19.75	49.15	-
10	47.3	40.25	8.20	45.15	0.68
Mean	45.0	33.51	13.68	39.85	0.62
STDV	2.7	7.76	11.03	11.46	0.30

4.4.2 Seasonal variation in carbohydrate concentration in pecan organs

Annual carbohydrates in deciduous trees are characterised by a depletion of the resources with the early growth followed by an accumulation period. In our study, soluble sugars (Figure 4.6), starch (Figure 4.7) and TNSC (Figure 4.8) reached a maximum in early winter and decreased in late winter/early spring.

Soluble sugars did not show a significant difference between the roots and trunk. Starch was the primary storage carbohydrate in both the trunk and roots. Starch concentration followed a different pattern between organs for several months. The only significant difference between organs was found for December when the values in the roots were significantly lower than those found in the trunk.

TNSC were greater during winter and declined to low levels in spring, coinciding with the time the tree depended on storage reserves until the leaves developed (Figure 4.8). Leaf TNSC fluctuated throughout the season, generally decreasing with a slight increase at the end of the season before senescence (

Figure 4.9).
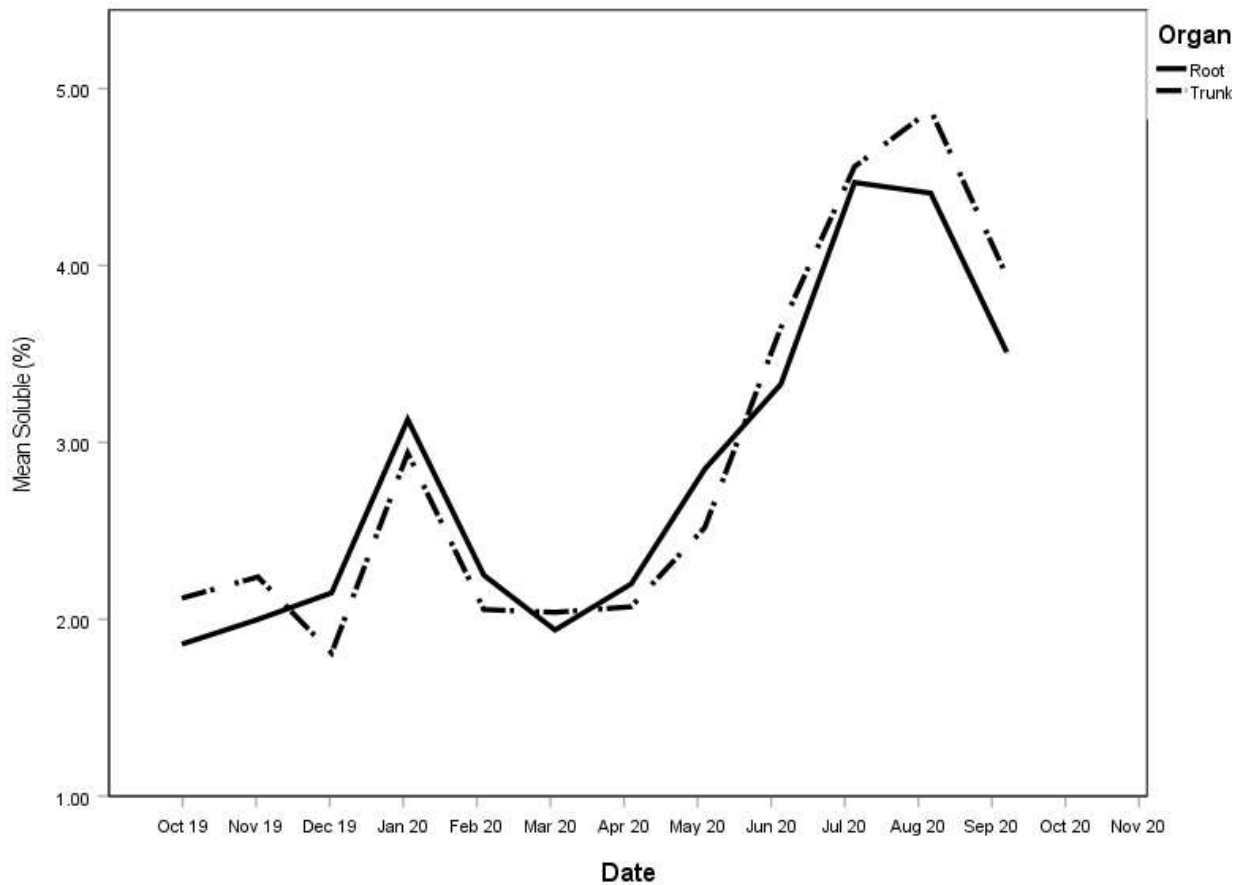
Figure 4.6: Seasonal variations in soluble sugars (SS) in roots and trunk.
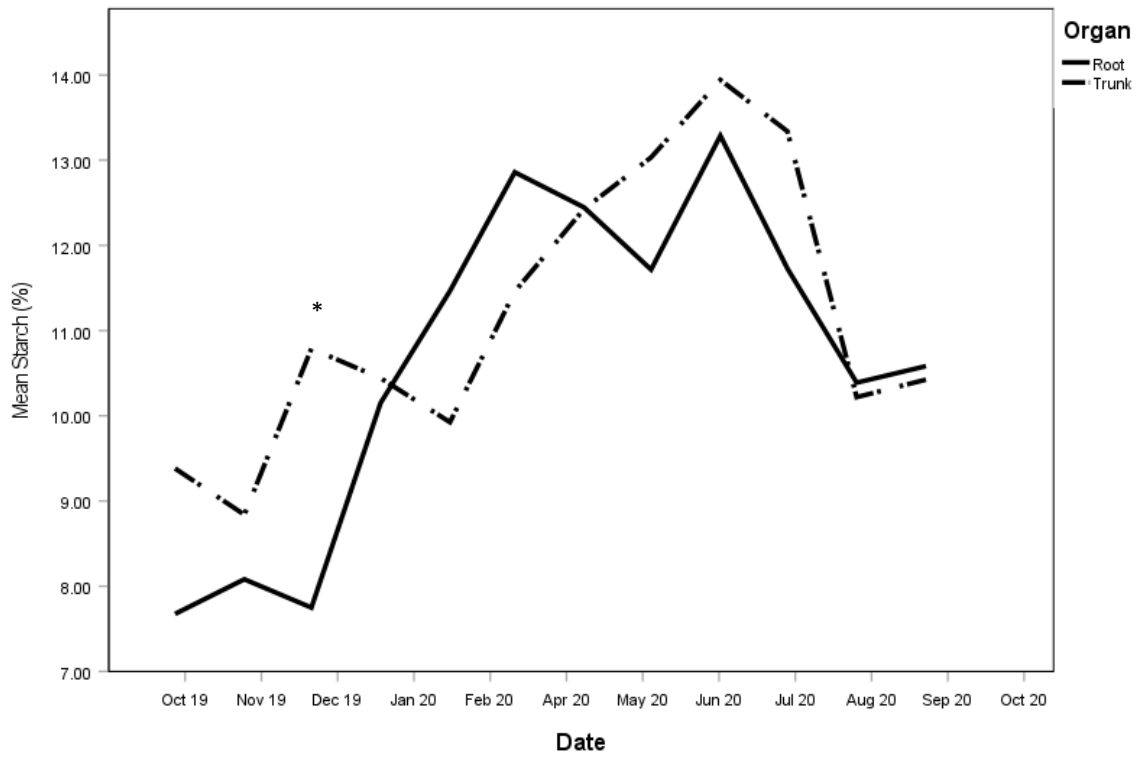
Figure 4.7: Seasonal variations in starches (%) in roots and trunk. * indicates significantly different values amongst organs (*, $p < 0.05$)
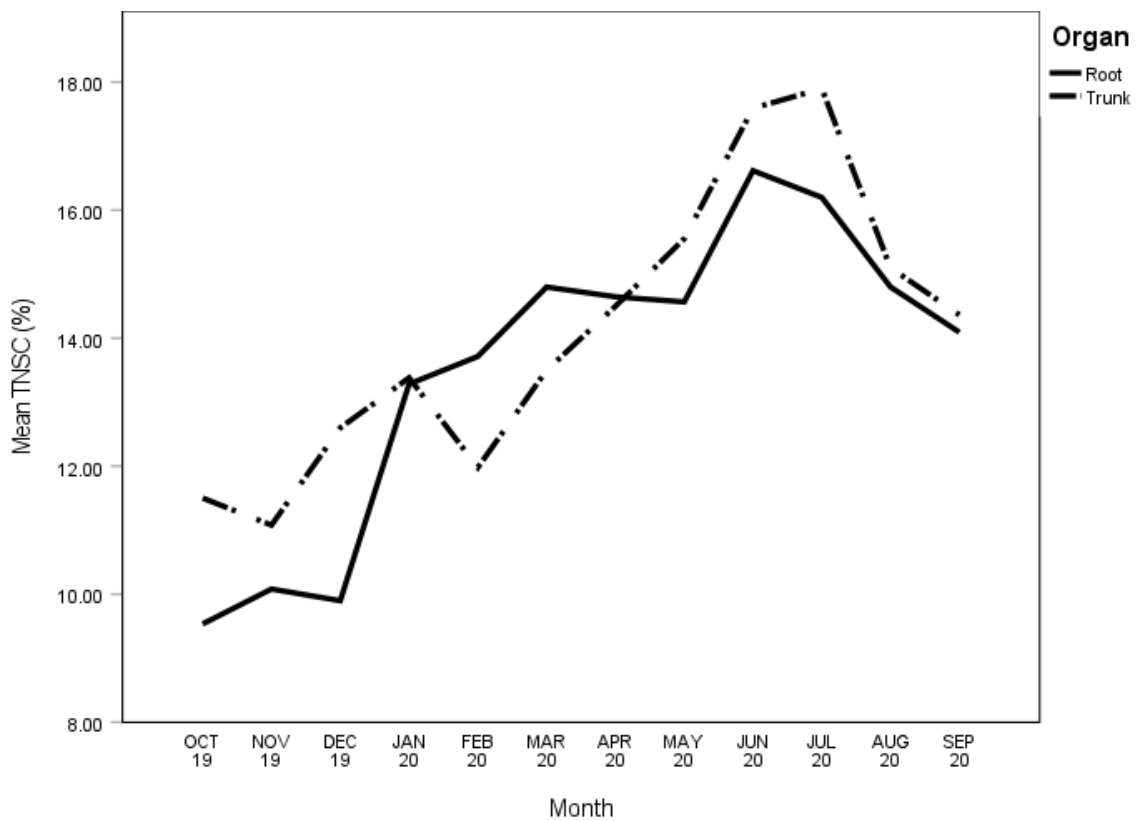
Figure 4.8: Seasonal variations in TNSC (%) in roots and trunk
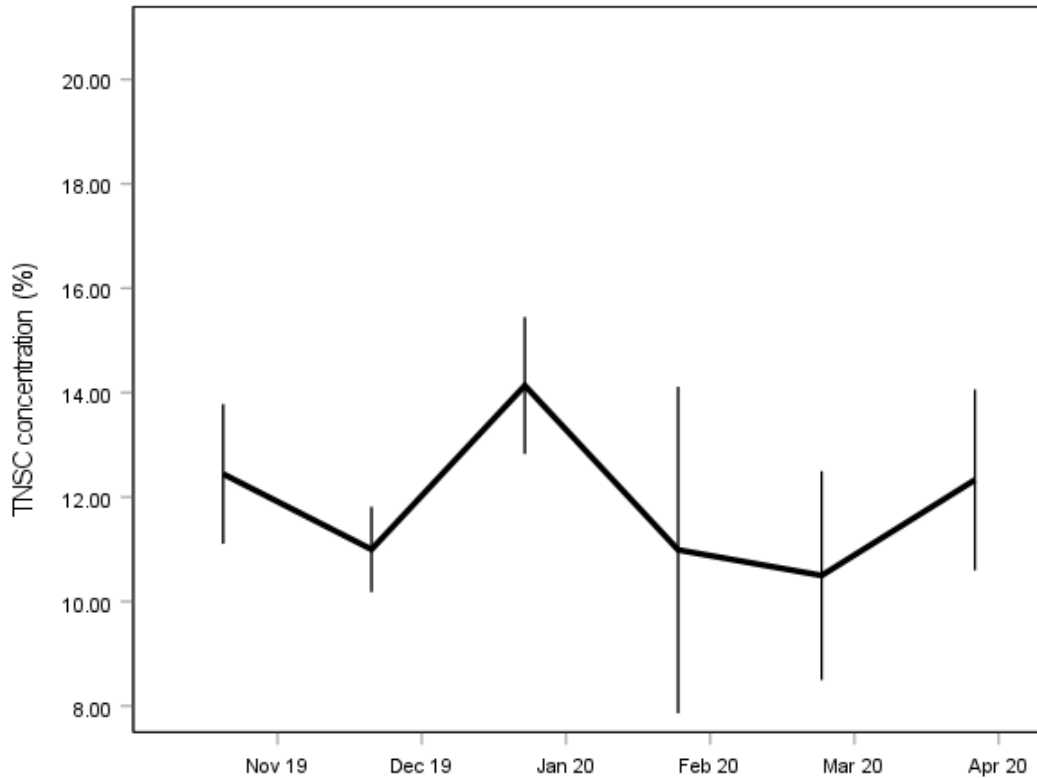
Figure 4.9: Seasonal variations in TNSC (%) in leaves

4.4.3 Nitrogen

In this study, nitrogen content in the leaves was higher than that in the other organs, and followed a negative gradient throughout the season. Leaf nitrogen declined from November to January, levelling off in February and continued to decline for the rest of the season. Month to month changes were not statistically significant (Table 4.2).

Patterns of nitrogen in the roots and trunk were not significantly different from each other. Nitrogen in the roots and trunk reached a minimum in December, reflecting the translocation and near depletion from budburst to the end of pollination, highlighting intensive vegetative activity at this time. The second drop in nitrogen for all organs was observed in April coinciding with the end of kernel fill. After kernel fill, nitrogen increased significantly to the first peak in May. A second peak was observed in July, and after that the values levelled off, showing no significant changes during dormancy.

Table 4.2: Seasonal changes in N composition for variety Wichita leaves, trunk and roots

	Mean percentage N composition		
Date	Leaves	Trunk	Roots
Oct-19	-	0.42	0.50
Nov-19	2.51	0.29*	0.42
Dec-19	2.49	0.26	0.37
Jan-20	2.41	0.33*	0.38
Feb-20	2.43	0.33	0.42
Mar-20	2.38	0.29	0.38
Apr-20	2.26	0.24	0.31
May-20	-	0.42*	0.50*
Jun-20	-	0.31*	0.34*
Jul-20	-	0.43*	0.43*
Aug-20	-	0.41	0.44
Sep-20		0.39	0.41

*The mean difference is significant at the 0.05 level to the previous month, P<0.05
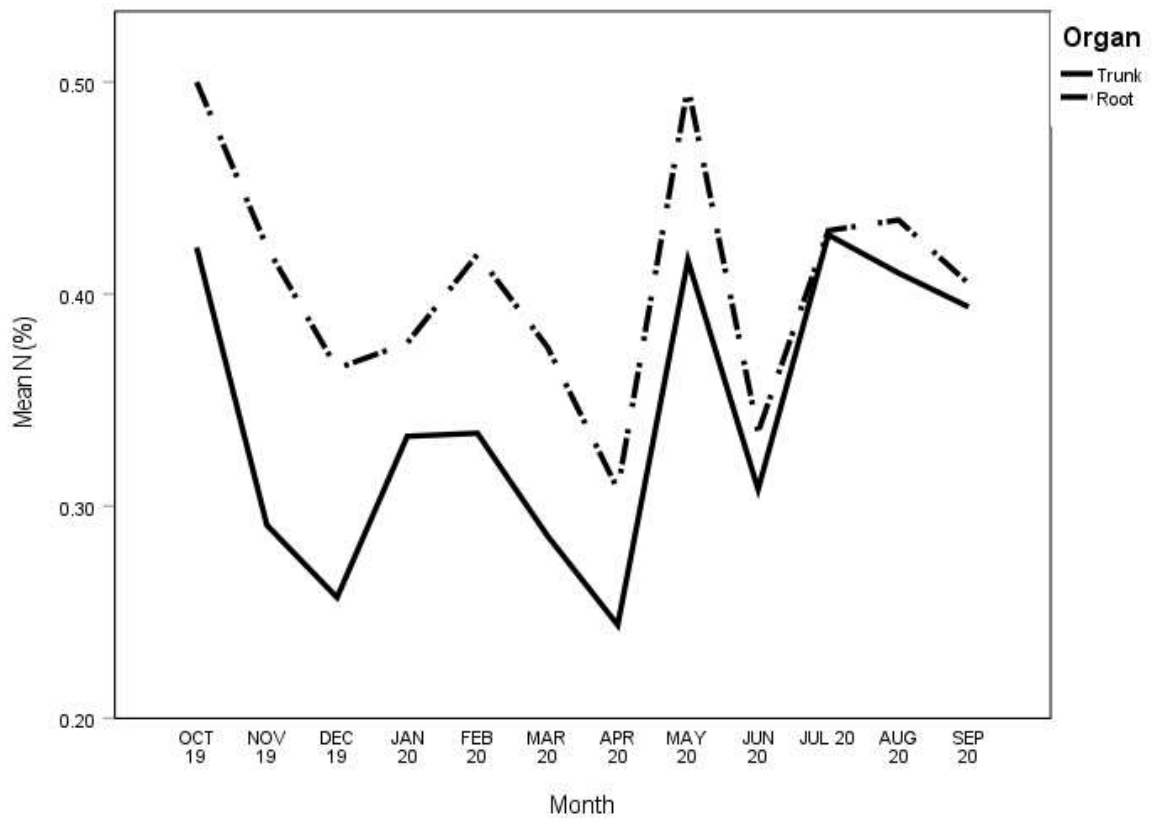
Figure 4.10: Seasonal fluctuations in N concentration for the different wood samples
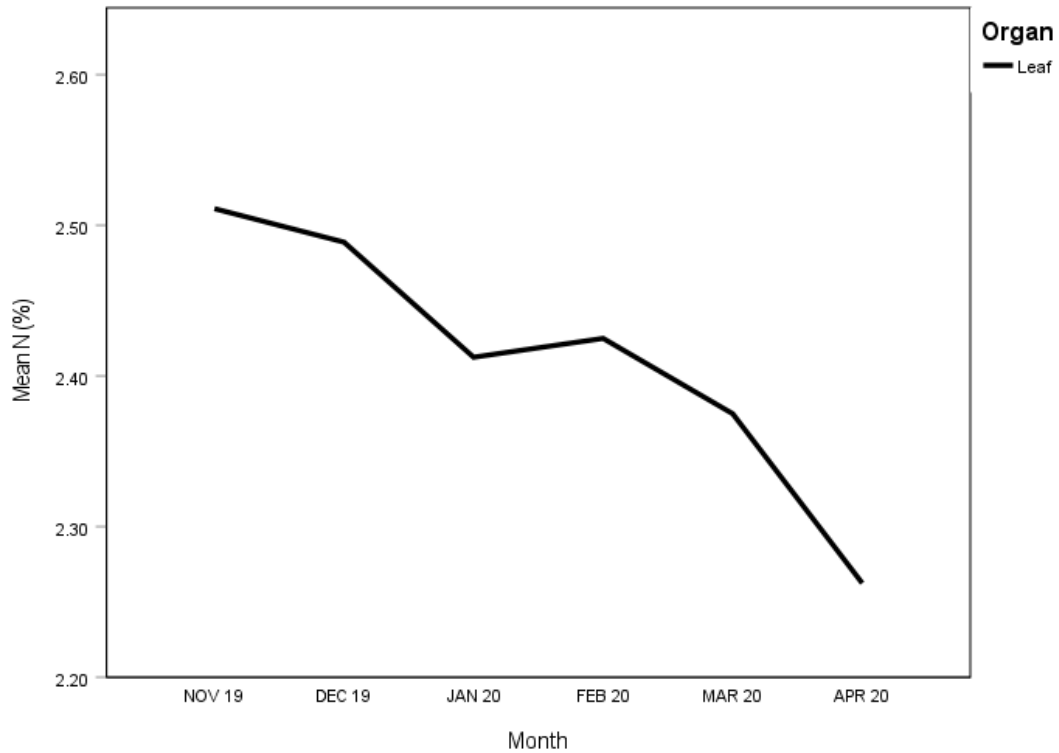
Figure 4.11: Seasonal variations in total non-structural N (%) in leaves

4.4.4 Phosphorous

Phosphorous concentration was lower than the other macronutrients analysed in this study. Like nitrogen, phosphorous declined throughout the season. Highest leaf concentration was obtained in December. After this peak, concentration declined the following month and was stable for the remainder of the season.

In the roots and trunk, no significant differences were observed throughout the season.

Table 4.3: Seasonal changes in P composition for variety Wichita leaves, trunk and roots

Date	Mean percentage P composition		
	Leaves	Trunk	Roots
Oct-19		0.02	0.02
Nov-19	0.19	0.03	0.02
Dec-19	0.13*	0.03	0.02
Jan-20	0.14	0.03	0.02
Feb-20	0.11*	0.02	0.03
Mar-20	0.15*	0.03	0.03
Apr-20	0.16	0.03	0.03
May-20		0.03	0.02
Jun-20		0.03	0.03
Jul-20		0.03	0.03
Aug-20		0.03	0.03
Sep-20		0.03	0.03

*The mean difference is significant at the 0.05 level to the previous month, $P < 0.05$
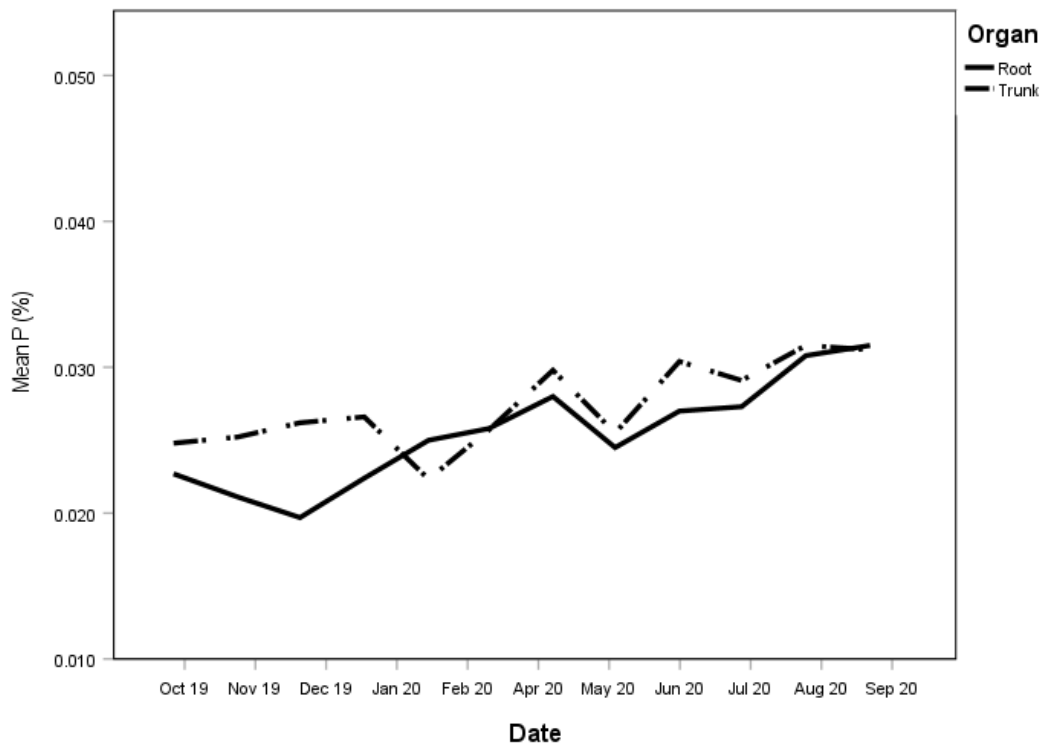
Figure 4.12: Seasonal fluctuations in P concentration for the different wood samples
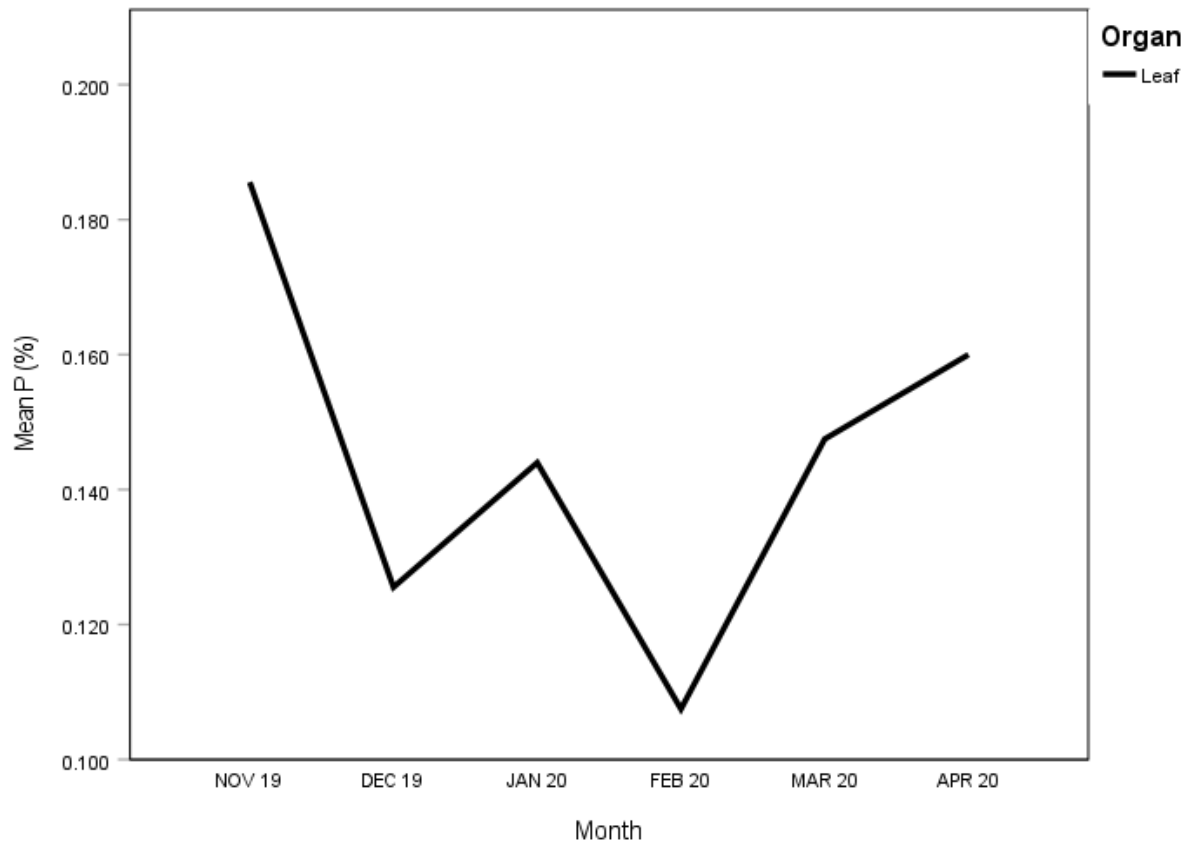
Figure 4.13: Seasonal variations in total non-structural phosphorous (%) in the leaves

4.4.5 Potassium

Potassium in the leaves followed a negative trend similar to the patterns for nitrogen and phosphorous. In leaves, the values decreased as the season advanced, with a minimum value during February. In the roots, changes were not statistically significant except for a decrease in July. In the trunk, a peak was achieved during November, after that it steadily decreased until February, levelling off until September (Table 4.4).

Table 4.4: Seasonal changes in K composition for variety Wichita pecan trees in leaves, trunk and roots

Date	Mean percentage K composition		
	Leaves	Trunk	Roots
Oct-19		0.14	0.15
Nov-19	1.23	0.17*	0.15
Dec-19	1.03	0.16	0.17
Jan-20	1.11	0.15	0.17
Feb-20	0.65*	0.12*	0.15
Mar-20	0.93*	0.12	0.13
Apr-20	0.95	0.12	0.14
May-20		0.11	0.14
Jun-20		0.10	0.12
Jul-20		0.11	0.15*
Aug-20		0.10	0.13
Sep-20		0.12	0.15

*The mean difference is significant at the 0.05 level to the previous month
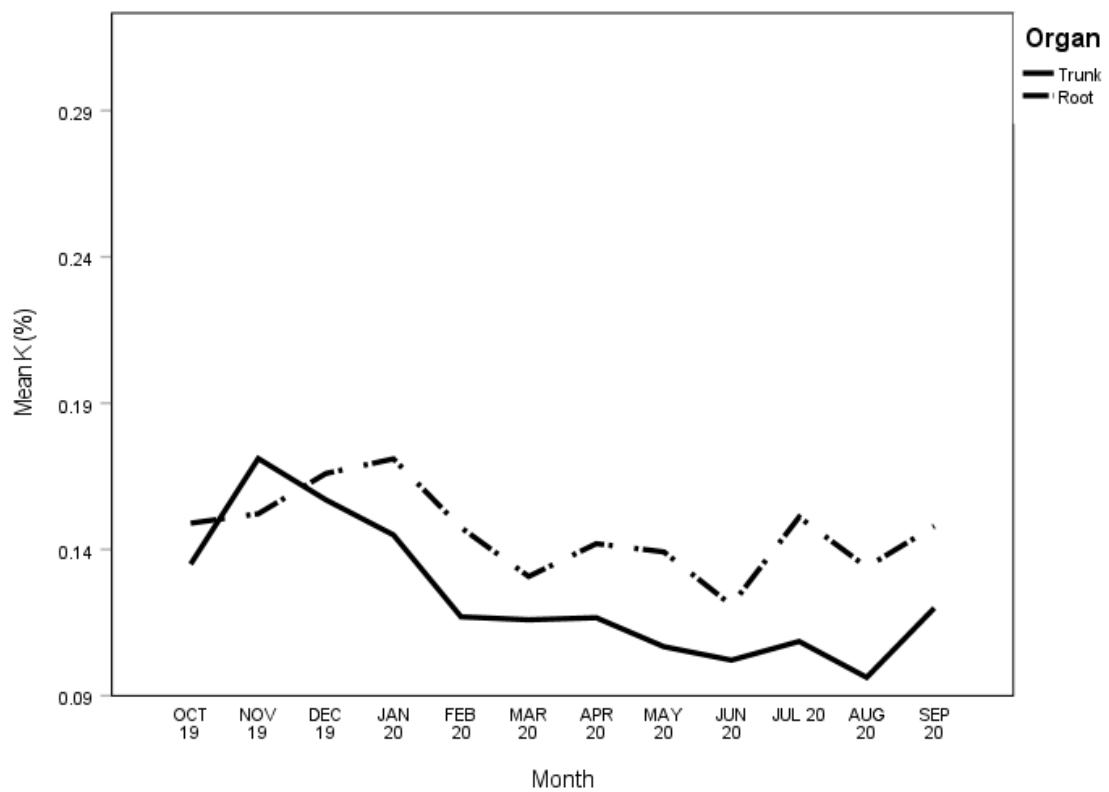
Figure 4.14: Seasonal fluctuations in K concentration for the different wood samples
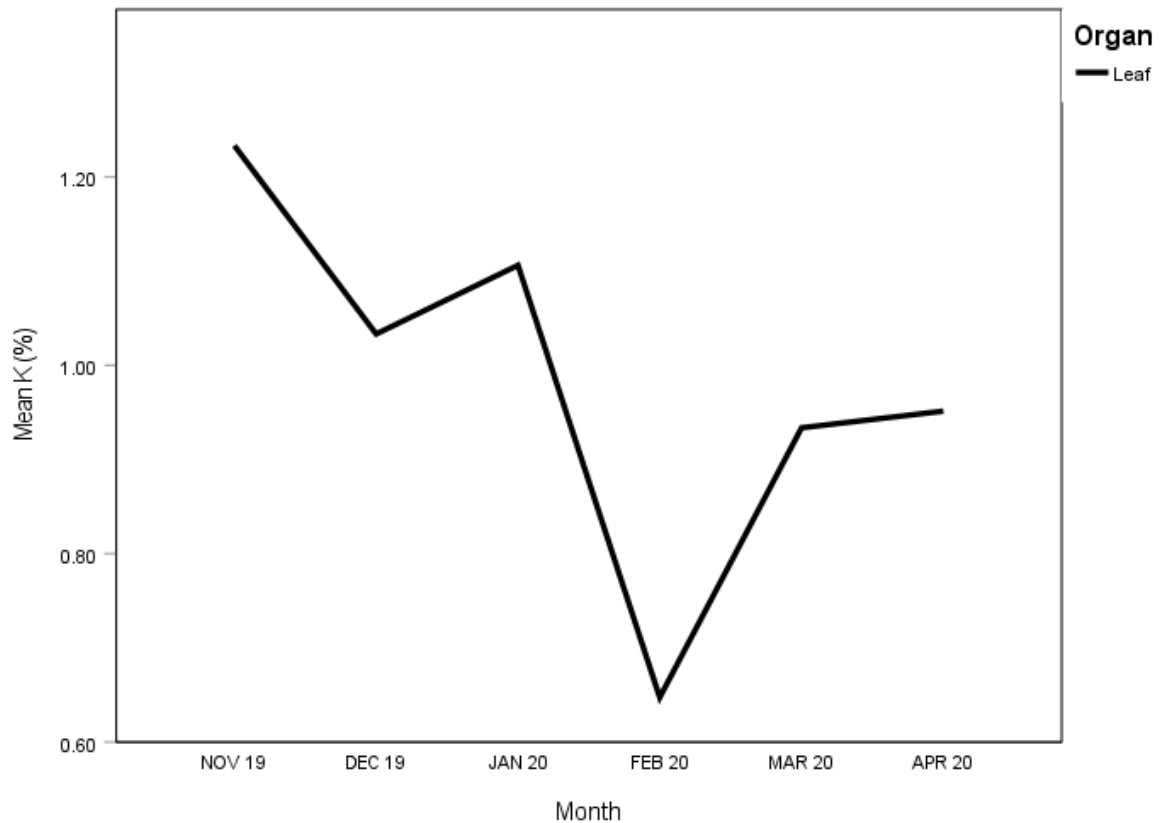
Figure 4.15: Seasonal variations in total non-structural K (%) in the leaves

4.4.6 Correlations of nitrogen, phosphorous, potassium and TNSC in leaves with yields

A moderate to fairly strong negative correlation was found between the 2020 yields and leaf TNSC, nitrogen and phosphorous sampled in November 2019 (Table 4.5). This interaction was significant to a level $P< 0.05$ for nitrogen (Figure 4.16 and Figure 4.17) and $P< 0.01$ for P (Figure 4.18). No significant correlations were found between leaf potassium and yields for the same month (Table 4.5).

Another statistically significant correlation was seen in the relationship between TNSC in the leaves in March, and end of kernel development with the yields harvested in May in the same year.

In most instances, no correlation was found between leaf analysis with previous or posterior yields, suggesting that leaf testing for nutritional levels is a good indicator only for the current crop year's tree health.

Table 4.5: Coefficients of correlation between TNSC, N, K and P concentration in the leaves for 2019, 2020 and 2021 yields

| | Coefficient of correlation between TNSC, N, K, P from November 2019 to April 2020 with 2019, 2020 and 2021 yields | | | | | | | | | | | |
| | TNSC | | | N | | | K | | | P | | |
Month	2019	2020	2021	2019	2020	2021	2019	2020	2021	2019	2020	2021
Nov-19	0.669	-.779*	0.463	-0.091	-.668*	0.036	-0.535	0.119	-0.046	0.270	-.830**	0.223
Dec-19	0.389	-0.269	0.545	-0.388	0.116	-0.569	-0.267	-0.225	0.024	-0.267	-0.225	0.024
Jan-20	-0.715	0.265	-0.457	-0.062	-0.451	-0.087	-0.553	0.037	-0.433	-0.089	-0.332	-0.175
Feb-20	-	-	-	-	-	-	-	-	-	-	-	-
Mar-20	0.191	-.794*	0.551	-0.247	-0.452	0.029	0.613	-0.543	0.473	0.608	-0.071	0.274
Apr-20	0.697	-0.646	0.515	0.473	-0.395	0.451	0.473	-0.395	0.451	0.724	-0.310	0.394

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

- N<7
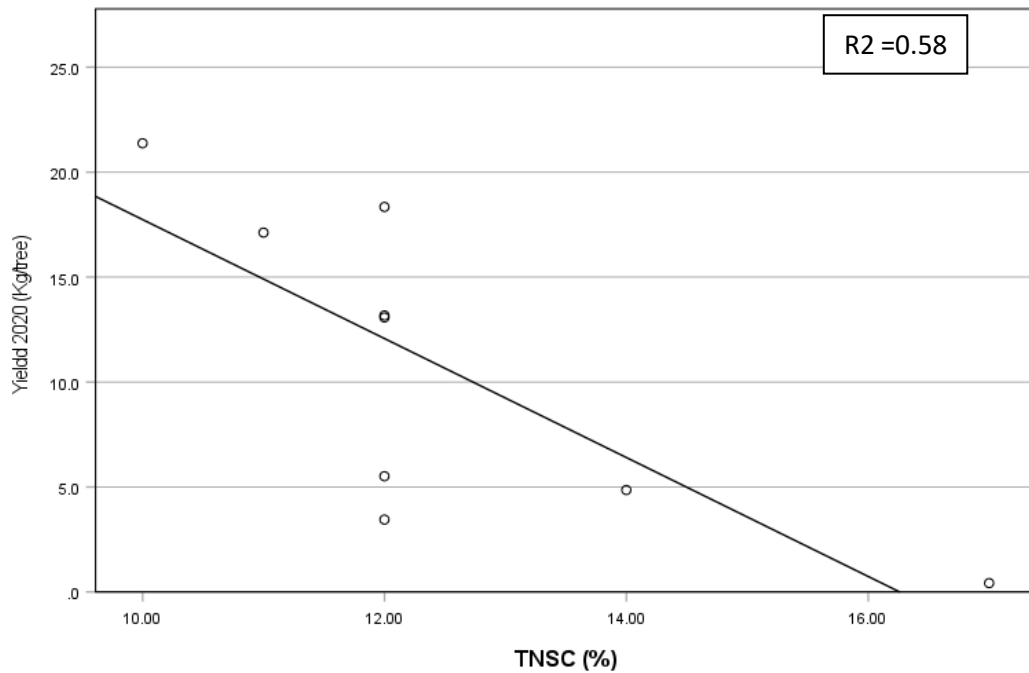
Figure 4.16: Pecan tree yield (kg/tree) related to the TNSC (%) in the leaves for November n=9, y= 46.08-2.83*x, R^2 = 0.58, P<0.05; where x is the TNSC (%) in the leaves for November 2019 and y is 2020 yields
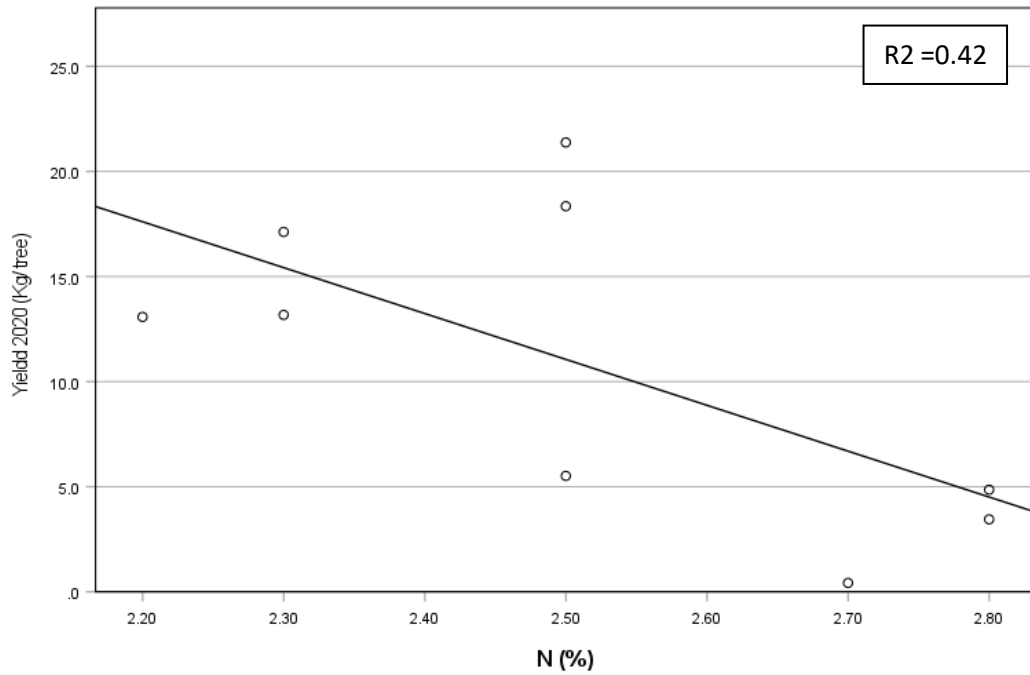
Figure 4.17: Pecan tree yield (kg/tree) related to N % in the leaves for November n=9, y= 65.62-21.82*x, R2 = 0.42, P<0.05; where x is the % TNSC in the leaves for November 2019 and Y is 2020 yields
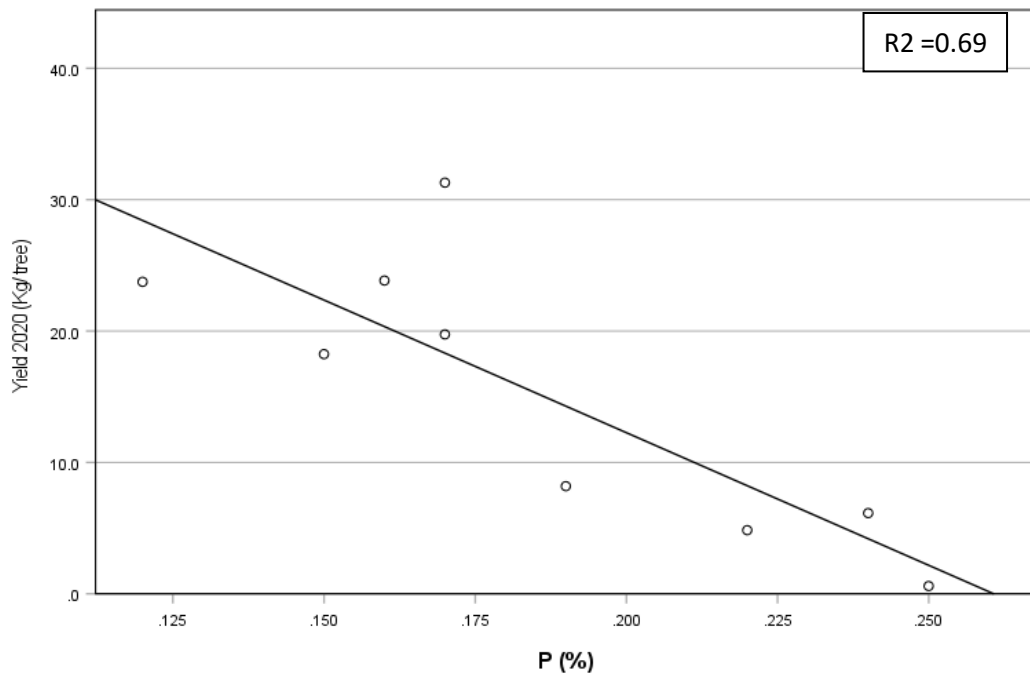
Figure 4.18: Pecan tree yield (kg/tree) related to the P % in the leaves for November n=9, Y= 52.65-2.02E2*x, R2 = 0.69, P<0.01; where x is the % TNSC in the leaves for November 2019 and Y is 2020 yields

4.4.7 Correlations of TNSC in trunk and roots with yields

There was only one positive correlation between TNSC in the trunk between September 2020 and subsequent yields (Table 4.6). This correlation indicates that the carbohydrate pool affected the yields at the time of bloom. However, the previous yields did not affect the carbohydrate pool available during bloom.

TNSC in the roots was rarely related to yields except for the carbohydrate content during March and April 2020 (shuck split) with 2021 yields. This correlation was positive in both instances. A second relationship was found between the carbohydrate concentration in the roots during January 2020 (nut sizing) and the same year's harvest. Production intensity had no significant effect on TNSC concentration in the trunk or roots (Table 4.6).

Table 4.6: Coefficients of correlation between TNSC concentration in the trunk and roots with 2019, 2020 and 2021 yields

	Coefficient of correlation between TNSC with 2019, 2020 and 2021 yields					
	Trunk			Roots		
Month	2019	2020	2021	2019	2020	2021
Oct-19	0.026	-0.091	0.129	-0.330	0.297	-0.320
Nov-19	-0.090	-0.202	0.519	0.504	0.148	-0.009
Dec-19	-0.277	-0.086	0.376	-0.199	0.134	-0.106
Jan-20	-0.113	-0.040	0.488	-0.556	.763*	-0.214
Feb-20	-0.119	-0.208	0.628	0.155	-0.385	0.582
Mar-20	0.089	-0.074	0.522	0.463	-0.309	.786**
Apr-20	0.185	-0.304	0.460	0.621	-0.501	.636*
May-20	-0.234	-0.159	0.591	-0.390	-0.135	-0.053
Jun-20	-0.018	-0.435	0.440	-0.434	-0.252	0.532
Jul-20	0.403	-0.371	0.425	0.270	-0.023	0.175
Aug-20	0.263	-0.349	0.483	-0.011	-0.173	0.192
Sep-20	0.119	-0.273	.658*	0.362	-0.165	0.352

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

4.4.8 Correlations of nitrogen, phosphorous and potassium in trunk and roots with yields

The percentage of nitrogen in the trunk for September was negatively correlated to previous yields and positively correlated to the following season, suggesting that nitrogen reserves are impacted by the previous crop size and impact the subsequent crop size. A similar relationship was found for November where nitrogen concentration was positively correlated with previous yields and negatively correlated with following yields (Table 4.7).

 Other correlations seem to be casual relationships.

Table 4.7: Coefficients of correlation between N, K and P concentration in the trunk with 2019, 2020 and 2021 yields

Coefficient of correlation between N, K and P concentration from November 2019 to April 2020 and 2019, 2020 and 2021 yields in the trunk									
	N			K			P		
Month	2019	2020	2021	2019	2020	2021	2019	2020	2021
Oct-19	0.110	-0.501	0.036	-0.216	-0.188	0.330	-0.022	-0.543	0.483
Nov-19	.802*	-.707*	0.361	0.113	-0.073	0.459	.832*	-0.213	0.601
Dec-19	-0.482	0.019	0.332	-0.365	0.026	0.147	0.005	-0.027	0.306
Jan-20	-0.246	-0.420	0.356	-0.083	-0.153	0.370	0.332	-0.339	.687*
Feb-20	0.408	-.679*	0.304	0.382	-0.457	0.389	0.320	-0.303	0.549
Mar-20	0.586	-0.355	0.209	0.394	-0.381	0.346	0.602	-0.198	0.441
Apr-20	0.357	-0.140	0.340	-0.430	-0.219	0.308	0.467	-0.089	0.514
May-20	0.067	-0.328	0.220	0.094	-0.177	0.412	-0.109	-0.204	0.213
Jun-20	-0.122	-0.223	0.466	0.052	-0.365	0.216	0.183	-0.003	0.319
Jul-20	0.023	-0.431	0.435	-0.264	0.201	-0.621	0.543	-0.106	0.147
Aug-20	-0.043	-0.409	0.465	-0.033	-0.254	0.014	0.352	-0.166	0.256
Sep-20	0.245	-.869**	.640*	-0.014	-0.387	-0.072	0.574	-0.169	0.070

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 4.8: Coefficients of correlation between N, K and P concentration in the roots with 2019, 2020 and 2021 yields

Coefficient of correlation between N, K and P concentration from November 2019 to April 2020 and 2019, 2020 and 2021 yields in the trunk								
	N			K			P		
Month	2019	2020	2021	2019	2020	2021	2019	2020	2021
Oct-19	-0.490	-0.051	0.113	-0.636	-0.285	-0.086	0.005	-0.274	-0.067
Nov-19	0.121	-0.397	0.588	-0.495	0.431	-0.192	-0.093	0.141	0.060
Dec-19	-0.113	-0.257	0.288	-0.264	-0.085	-0.314	-0.037	-0.347	0.122
Jan-20	-0.054	0.324	0.116	0.694	-0.213	0.312	-0.001	-0.196	0.344
Feb-20	0.565	-0.344	0.064	-0.079	0.223	0.450	0.534	-0.467	0.131
Mar-20	0.110	-0.301	0.533	-0.507	0.319	0.065	0.552	-0.289	0.530
Apr-20	0.152	0.017	0.221	0.311	0.067	0.373	.717*	-0.411	0.443
May-20	-0.326	-0.007	0.114	-0.553	0.113	0.199	-0.647	0.108	-0.128
Jun-20	-0.010	-0.297	0.408	0.479	-0.106	0.501	0.168	-0.329	0.419
Jul-20	0.528	-0.205	0.332	0.271	0.127	-0.134	0.274	0.289	-0.251
Aug-20	-0.026	-0.207	0.269	0.143	0.054	0.355	0.167	-0.191	0.240
Sep-20	0.254	-0.369	0.353	0.694	-0.296	0.227	0.563	-0.160	0.351

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

4.5 DISCUSSION

This work presents an integrated evaluation of seasonal patterns in soluble carbohydrates, starches and nutrients of pecan trees over a season. Starch was the main form of carbohydrate present in the samples. It followed a different pattern for organs over several months however, the only significant difference between organs was found for December when the values in the roots were significantly lower than those found in the trunk. Soluble sugar concentrations did not show a significant difference between the roots and trunk.

As carbohydrates are readily converted, the concentration of individual carbohydrates is less important than TNSC (Tromp 1983). Seasonal patterns of TNSC were similar to those reported in Chapter 3 and consistent with the results reported in previous research (Smith & Waugh 1938; Krezdorn 1955; Worley 1979a). Generally, TNSC were greater during winter and declined to low levels in spring when the tree depended on storage reserves. The spring to early summer minimum occurred as a consequence of primary and secondary growth where the winter minimum was associated with the hardening process (Worley 1979a).

 Previous research in carbohydrates in different organs of the tree reported differences between the carbohydrate content of the roots and trunk (Kozlowski 1992; Briceño Contreras et al. 2018). In this study, the values obtained for trunk and roots were, in the majority of cases, not significantly different. We believe that this is due to sampling taking place in the lateral exposed root rather than fine to medium size roots below ground that were the subject of other studies. Leaf TNSC fluctuated throughout the season, generally decreasing with a slight increase at the end of the season before senescence.

Krezdorn (1955) indicated a decreasing gradient for nitrogen, phosphorous and potassium as the season advances. Our research showed similar patterns. The major period of nitrogen accumulation in pecans occurs as new leaves, shoots and flowers are developing in the spring (Acuña-Maldonado et al. 2003). The concentration of nitrogen in the leaves was highest at the start of the season following a negative trend until the end of the season. Phosphorous and potassium had two distinct valleys in the leaf results. The first was in December coinciding with the nut set and the second during kernel fill in February. Both of

these phenological stages are very demanding. Fruit is considered a strong sink due to its heavy demand and rapid metabolic activity (Kim & Wetzstein 2005). Also, Krezdorn (1955) observed a sharp drop in phosphorous in summer, speculating that this drop was a reflection of phosphorous drain during flowering.

Nitrogen is a primary component of fertility management in pecans. Early spring growth, flowering and embryo development use current nitrogen inputs and stored nitrogen resources (Kraimer et al. 2001). In our study, the roots and trunk showed the impact of rapid spring growth and nut filling on nitrogen stores. The demand that kernel filling presents to the crop was reflected in the drop in nitrogen observed in March. The depletion of nitrogen during kernel fill for a heavy crop year may reduce the reserves available for the following season (Kraimer et al. 2004), thus promoting alternate bearing. Potassium in the trunk peaked during November, and after that it steadily decreased until February, levelling off until the end of the season. Phosphorous showed no consistent seasonal variations in the trunk or roots.

The most consistent relationships were found for September before budburst and the month of November; a period coinciding with flowering. Other correlations found randomly through the data could be the result of causal relationships.

The correlations obtained between leaves and yields presented different degrees of linear association for each element. A fairly strong negative correlation was found between the leaf TNSC, nitrogen and phosphorous sampled in November, coinciding with the end of pollination; the start of nut sizing with subsequent yields. Since this correlation is negative, it can be hypothesised that rather than those elements being the limiting factors, the translocation of elements to the fruiting parts of the trees is of importance as the developing fruit is a strong sink. Further evaluation is recommended.

The percentage of nitrogen in the trunk for September was negatively correlated to previous yields and positively correlated to the following season, suggesting that nitrogen reserves were impacted by the previous crop size and impacted the subsequent crop size. A similar relationship was found for November where nitrogen concentration was positively correlated with previous yields and negatively correlated with following yields.

Research done in grapevines, determined that the status of nitrogen available early in the season could determine fruit yield and growth (Cheng et al. 2004).

Trunk TNSC in September, at the time of budburst, was related to subsequent yields. This correlation indicates that the carbohydrate pool affected the yields at the time of bloom and, as previous research has pointed out, the potential for carbohydrates to be the limiting factor. These results are consistent with the outcomes presented by Wood (2014). He pointed out the relationship between storage sugars made available to the tree during the transitional period when buds are swelling, with subsequent yields. This research also supports previous findings where no correlation was found between carbohydrates, potassium or nitrogen in winter with the subsequent yields (Smith et al. 2007).

Although this research does not answer the question as to whether the carbohydrates are the sole factor for alternate bearing and yielding potential of trees, it does highlight the potential for carbohydrates to become part of the monitoring regime in farms to assess tree health and to aid crop forecasting. Routine measuring of carbohydrates in the orchard, perhaps by selecting representative trees, could improve yield predictions. Further work to refine the relationship between carbohydrates and subsequent yields is paramount

CHAPTER 5 : EFFECT OF PRUNING SEVERITY AND TIMING IN PECAN TREES ON CARBOHYDRATE CONTENT AND YIELD

5.1 ABSTRACT

The influence of pruning timing, type and severity on pecan trees was assessed to investigate the effects on the carbohydrate pool and subsequent yields. The experiment was carried out at the Trawalla farm in New South Wales, Australia on 50-year-old variety Wichita pecan trees. Trees were pruned at different times and using different methods: (1) heavy pruning early winter, (2) heavy pruning late winter, both sides of the trees pruned (approximately 40 % above ground biomass removed) , (3) light pruning, only one side of the tree (approximately 20 % above ground biomass removed)and (4) unpruned, (5) root pruning. None of these pruning methods altered TNSC concentration in the trunk.

Yields were generally higher in the control treatment and root pruning, however the difference was not statistically significant. There were no significant differences for treatments in TNSC or tree growth.

Pruning has been suggested as a management practice to alleviate alternate bearing, but our results lead to the conclusion that pruning by itself might not be enough to remedy alternate bearing in pecan trees. On the other hand, it is worth noting that as pruning is required to reduce overcrowding and improve light interception, pruning going into an "on" year is favourable as the impact on crop and reserves is minimal. Further research to quantify the impact of pruning in the carbohydrate pool is required.

5.2 INTRODUCTION

Stored carbohydrates play a critical role in all trees. Pecan trees, like any other deciduous tree, transport and store carbohydrates to different organs to support growth. Early growth in spring, yield efficiency and productivity are largely influenced by the reserve store and availability during dormancy. During the period of dormancy, carbohydrates are

stored in the perennial organs (Glerum 1980; Tromp 1983; Oliveira & Priestley 1988; Cheng & Robinson 2004; Da Silva et al. 2014).

Changes in carbohydrate reserves can impact spring regrowth (Worley 1979a), and pruning is one of the activities that can affect carbohydrate reserves (Khan et al. 1998). Pruning is an important management practice in the pecan industry. Pecan trees are naturally forest trees that must compete for light. They exhibit apical dominance; meaning they put much of their energy towards the tips that are receiving the most light. This ensures that they can quickly dominate the light within the forest, and thus out-compete their neighbours. In nature, the trees will shade out their lower limbs which will eventually be lost (Sparks 2005). So, it makes sense for a productive well-managed orchard to periodically prune to improve light interception as pecan yields appear to be limited by shading. Hedge pruning and topping are also recommended to improve spray coverage. Previous studies have confirmed the benefits of pruning (Heerema et al. 2016; Wells 2018).

Pruning at Trawalla, is usually performed during the dormancy period. Depending on the climate during pruning and the number of hectares to cover, the operation can take months. On some occasions, however, pruning is completed within weeks before budburst. Since carbohydrates are mobilized in deciduous trees to support spring growth, this research aims to explore whether the timing and severity of pruning could impact the carbohydrate reserve and subsequent yields. And if so, what would the recommended best practices for pruning be?

Hence, the overarching aim of this work was to evaluate the different pruning times, severity and methods according to their effects on the carbohydrate pool and yields.

5.3 MATERIALS AND METHODS

This experiment was carried out at Trawalla in New South Wales, Australia on 50-year-old variety Wichita pecan trees from the same flood irrigated field (Figure 5.1).

The experiment was arranged as a completely randomized block design, with four replicates per treatment and five trees on each treatment. Each experimental block

coincided with one irrigation step (Table 5.1). The treatments consisted of: (1) heavily pruned early in the dormancy in July, pruning both sides of the trees at 12 m, (2) heavily pruned late in the dormancy in September, (3) one half of the tree pruned early season in July to 12 m, (4) unpruned and (5) root pruning early in the dormancy in July (Table 5.2 and Table 5.3). An untreated buffer row was left between each intervention row to avoid impact from the neighboring row's treatment that could obscure the results.

Pruning was done using a mechanical pruner set at 10 m height (Figure 5.1). Root pruning was performed at 15 cm depth along both sides of the row, approximately 1 m away from the trunk.

The evaluated variables were subsequent yield and number of nuts. Due to late rainfall during harvest in 2021, we could not arrange for individual trees to be shaken and harvested separately. Instead, we determined an average weight of the trees by subsampling nuts lying on the ground in two cardinal directions: north and south. Tree rows ran east to west. The average of nuts weighed in the sampled areas were then used to estimate yield per tree.

Tree height and canopy diameter were obtained using LiDAR technology. A plane with a LiDAR sensor was flown in March 2021. The LiDAR model RIEGL VQ-780i was used to carry out the survey (Figure 5.3). LiDAR data captured a minimum of 20 points per square metre at a flying height of 1000 metres to ensure 10 centimetre vertical accuracy. LiDAR surface data has a fundamental vertical accuracy +/- 10cm at 1 sigma confidence interval (RMSE), on open flat hard ground, as measured against valid ground control.

TNSC was measured in the trunks of the trees following the method described in Section 3.3.3.1 Determination of TNSC. Data was analysed by two-way ANOVA for each treatment to test significance. Interactions between factors was considered significant at $P <= 0.05$.
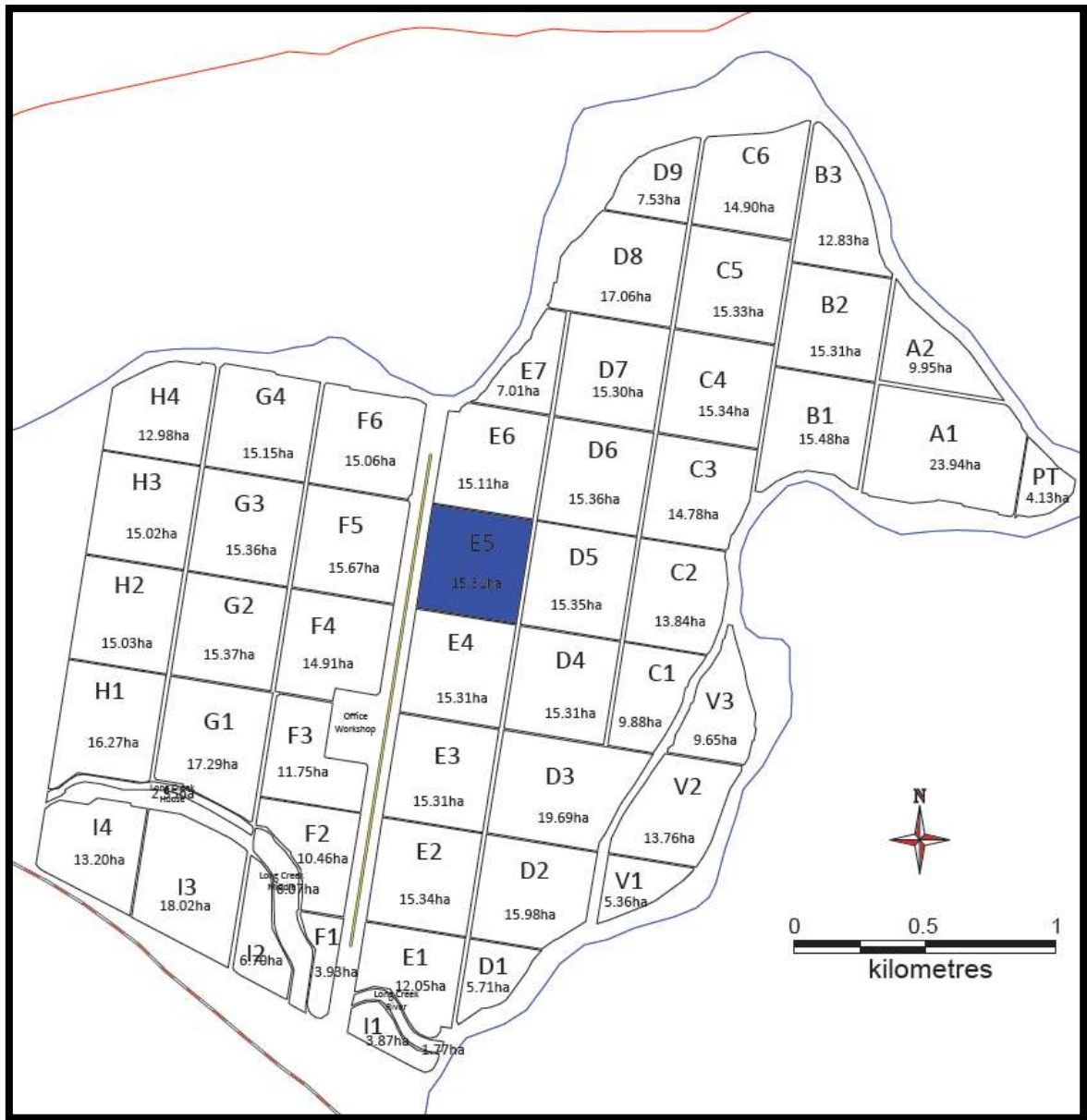
Figure 5.1: Trawalla farm showing field selected (in blue)

Figure 5.2: Mechanical pruner in field E5

Figure 5.3: Trimble unit used for farm flight surveying (March 2021)

Table 5.1: Experimental information

Farm	Trawalla
Block & variety	E5 Wichita
Trial design	Randomized complete block
Interventions	5
Replications	4
Total experimental area	15.3 ha
Tree spacing	10 meters
Row spacing	10 meters
Tree age	50
Irrigation type	Flood

Table 5.2: Pruning experiment treatments

Treatment	Description
A	Heavily pruned (pruning both sides of the tree) early in dormancy - July
B	Heavily pruned (pruning both sides of the tree) late dormancy - September
C	1/2 tree pruned early dormancy - July
D	Unpruned (control)
E	Root pruning early dormancy - July

Table 5.3: Pruning trial plot

Block	Block I					Block II					Block III					Block IV				
Wichita Rows	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40
Trial Row n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Treatment	A	E	B	C	D	A	D	C	E	B	D	A	C	B	E	D	A	C	E	B
1																				
2																				
3																				
.																				
.																				
.																				
38																				
39																				
40																				

5.4 RESULTS

There were no significant differences between the control and any of the pruning treatments for all the testing dates (Table 5.4). Root pruning gave the only significant reduction in carbohydrates between the heavy pruning treatments (early and late pruning). Overall higher yields were obtained in the control rows and for root pruning, and lower yields were obtained in the heavily pruned late trees, however these differences were not significant (Table 5.5).

The treatments did not seem to affect the rate of vegetative growth after pruning. Heavy pruning early versus late achieved similar mean heights. We found the same results when comparing control versus root pruning, as shown in Table 5.6.

Table 5.4: TNSC (%) in the trunk of pecan trees before and after pruning treatments that took place in August 2020 for early pruning and September 2020 for late pruning (n.s = not significant)

	TNSC (%) Testing date				
Treatment	Aug-20	Sep-20	Oct-20	Jan-21	Apr-21
Control	17.04	16.68	12.40	11.75	15.05
1/2 tree (13 Aug 2020)	16.58	16.71	12.50	12.55	14.85
Heavy early (13 Aug 2020)	16.73	16.42	12.45	11.35	13.70
Heavy late (17 Sep 2020)	16.53	16.61	12.70	12.15	13.90
Root pruning (13 Aug 2020)	16.48	16.10	12.20	12.95	16.10
Significance	n.s	n.s	n.s	n.s	P<0.01

Table 5.5: Average yields of pecan trees after pruning treatments (n.s = not significant)

	Yield
Treatment	Average weight (kg)
Control	11.1647
1/2 tree (13 Aug 2020)	9.5355
Heavy early (13 Aug 2020)	9.9021
Heavy late (17 Sep 2020)	8.9665
Root pruning (13 Aug 2020)	11.5687
Significance	n.s

Table 5.6: Mean canopy height of pecan trees obtained from LiDAR flight (n.s = not significant)

Treatment	Canopy Height Mean (m)
Root pruning	11.9485
Control	11.8952
	n.s
Heavy early	7.9497
Heavy late	7.7855
	n.s

5.5 DISCUSSION

For this study, we set out to examine the effect of pruning timing and severity on the trees' carbohydrate pool, yield and vegetative growth. A consideration to keep in mind when looking at the results is that the field chosen for this project had an extremely low yield in 2020 so, when the pruning was done during dormancy, all the fields had very high levels of storage carbohydrates.

The results show that pruning does not significantly affect the yield or carbohydrate pool when done in the dormancy before an "on" year. Pruning has been suggested as a management practice to alleviate alternate bearing, however our results lead to the conclusion that pruning by itself might not be enough to remedy alternate bearing in pecan trees.

This research only partially answers the question about the impact of pruning timing as assessment of the effect on pruning timing going into an "off" year still needs to be undertaken. These findings support management practices of pruning going into an "on", as it shows no significant difference between treatments. However, we don't know if we will get the same results doing the same experiment going into an "off" year.

Ideally this research needs to be done again having trees in both phases. To do so, we might need to artificially change the status of the trees by defoliating them the previous season.

CHAPTER 6 : GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The combined studies presented in this thesis had, as their main objective, the study of carbohydrates and nutritional aspects of pecan trees in Australia and their impact on yield, with an emphasis on determining whether carbohydrates could be further explored and used as a tree health assessment tool with the potential for the further use of this information for forecasting.

The scope of this study was:

(1) To study the seasonal variation of carbohydrates sampled monthly in the trunks of pecan trees and the potential relationship to yield

(2) To investigate TNSC, starch, soluble carbohydrates, nitrogen, phosphorous and potassium dynamics in the leaves, trunk and lateral roots over a 12 month period to identify seasonal patterns within the different organs of the pecan tree, and how yields could impact storage and vice versa (how resources determine subsequent yields)

(3) To study the impact of pruning timing and severity on the carbohydrate pool, subsequent growth and yield.

That concentrations of carbohydrates vary in response to the phenology of trees is well established. The data presented in Chapters 3 and 4 make a valuable contribution to the pecan field of research by providing seasonal measurements of carbohydrates and minerals in different tree organs. This thesis extends previous studies in seasonal changes in carbohydrates by comparing the values to final yields.

The seasonal patterns for both varieties considered in this research were similar to those reported previously (Smith & Waugh 1938; Krezdorn 1955; Worley 1979a). Total carbohydrate concentration in the trunk steadily decreased from spring to early summer, then increased to a peak in autumn, and slowly declined during winter. The minimum carbohydrate concentration was achieved in June, coinciding with the start of the

Australian winter. The winter minimum is believed to be caused by the conversion of starch to sugars during the hardening process (Worley 1979a).

While there were similarities in the seasonal patterns of the carbohydrates for the varieties studied, there were also significant differences in TNSC quantities. These differences could be related to the capacity of different varieties to harvest carbohydrates and their respective dates for budburst and defoliation (Wood et al. 2003). Generally, variety Wichita's budburst occurs one to three days before variety Western Schley, and it holds its foliage for a longer period when compared to variety Western Schley. This finding highlights the importance of adopting management initiatives that contribute to leaf retention after harvest.

The seasonal patterns in both varieties differed when segregated by years going into an "on" and "off" crop year. TNSC was higher in the "on" year fields from dormancy up to October, supporting the hypothesis that carbohydrate concentration is greater early in the season for "on" years (Wood & McMeans 1981; Nzima et al. 1997; Wood 2014). TNSC were generally higher in summer in "off" trees, and similar results have been obtained in other studies (Crane & Al-Shalan 1997). A minimum value of TNSC was obtained for fields going into an "on" year before dormancy. These results contradict the results obtained in the studies undertaken by Bustan et al. (2011), where "on" versus "off" carbohydrates in olives trees did not show any different patterns in carbohydrate concentrations.

This study also showed that the accumulation of carbohydrates in the trunk is influenced by temperature, solar radiation and rainfall. A significant moderate regression was obtained for the relationship between TNSC and temperature. Variety Wichita was found to accumulate a greater amount of carbohydrates than variety Western Schley. These results are similar to those of Briceño Contreras et al. (2019) for the relationship between starches in the root of both varieties and temperature. This result suggests the role of environmental cues in carbohydrate allocation throughout the season.

The literature concerning pecan alternate bearing determined that carbohydrate reserves play a minor role in alternate bearing pecan trees (Rohla et al. 2007). This study, however, suggests that there are moderate relationships between carbohydrates in the trunk in July

(dormancy) and subsequent yields, and a strong negative correlation with TNSC concentration in the trunk in October during flowering with the next year's crop. Interestingly, no association was found between the same month and current yields. Female flowers are known to be fueled by energy stored within the tree from the same year (Wells 2017), so we can conclude that this relationship might be casual.

When analysing the relationships of TNSC to yields in the trunk of an individual tree versus yields for one season (in Chapter 4), a similar negative correlation was found for September. It is worth noting that the year we conducted this experiment, budburst occurred two weeks earlier than in previous years. As we only sampled on the same day of each month, the equivalent month for that season would have been October. In the same scenario, no relationship was found for June (dormancy), contradicting the results obtained in Chapter 3.

The other major component of this thesis was to investigate the carbohydrates, starches and nutrients of pecan trees over a season. Starch was the main form of carbohydrate present in the samples. The values obtained in the trunk and roots were, in the majority of cases, not significantly different. We believe that this is due to sampling taking place in the lateral exposed root rather than the fine to medium size roots below ground that were the subject of other studies.

Krezdorn (1955) indicated a decreasing gradient in the concentration of nitrogen, phosphorous and potassium as the season advances. In general, our research shows similar patterns. The major period of nitrogen accumulation in pecan occurs as new leaves, shoots and flowers are developing in the spring (Acuña-Maldonado et al. 2003). The concentration of nitrogen in the leaves was highest at the start of the season following a negative trend until the end of the season. Phosphorous and potassium had two distinctive valleys in the leaf results. The first in December coinciding with the nut set, and the second during kernel fill in February. Both of these phenological stages are very demanding. Fruit is considered a strong sink because of its high demand and rapid metabolic activity (Kim & Wetzstein 2005). Krezdorn (1955) observed a sharp drop in phosphorous during summer, speculating that this drop was a reflection of the phosphorous drain during flowering.

A moderate to fairly strong negative correlation was found between leaf TNSC, nitrogen and phosphorous sampled in November; coinciding with the end of pollination and the start of nut sizing with subsequent yields. Since this correlation is negative, it can be hypothesised that rather than those elements being the limiting factors, what is of importance is the translocation of elements to the fruiting parts of the trees.

The final component of this work was to investigate how pruning severity and timing affected the carbohydrate pool, tree growth and yields. There were no significant differences in any of the study parameters for treatments. This research only partially answers the question about the impact of pruning timing. Whether there is an effect on pruning timing going into an "off" year must still be assessed.

The results show that pruning does not significantly affect the yield or carbohydrate pool when undertaken in dormancy before an "on" year. Pruning has been suggested as a management practice to alleviate alternate bearing, but our results lead to the conclusion that pruning by itself might not be enough to remedy alternate bearing in pecan trees.

6.1 CONCLUSIONS

In conclusion, although this research does not answer the question as to whether carbohydrates are the sole factor for alternate bearing and the yielding potential of pecan trees, it does highlight the potential for carbohydrates to become part of the monitoring regime on farms to assess tree health and aid crop forecasting.

The present study indicates that there is a moderate relationship between carbohydrates and subsequent crop. However, it is essential to note that these findings do not necessarily disagree with the role that phytohormones play in crop sets. Additionally, it is important to consider the stresses that a crop is under from the prediction date to harvest date. Hence, it is reasonable to hypothesise that fruit set, rather than the final crop, is dependent on the carbohydrate pool.

There is an opportunity for the pecan industry to continue research in this field to build a comprehensive yield forecasting tool using carbohydrate status combined with

environmental parameters. More research and data collection related to carbohydrates in pecans are warranted. Furthermore, we believe that the next step is to study how management practices on-farm could affect the carbohydrate pool.

6.2 FUTURE RESEARCH

From these conclusions, several recommendations for further studies can be made:

1. Investigations into a continuous monitoring system for carbohydrates. Development of a sensor that can detect sugars or the application of readily available sensors for this purpose. Continuous monitoring will aid in understanding not only the impact of seasonal patterns on carbohydrates, but how carbohydrates are translocated during the day

2. Sampling dates based on days after budburst rather than a fixed day so comparisons between years are meaningful. We noted that during the period of this study, budburst date varied from one season to the next

3. Pruning time and severity studies going into an "off" year. This study was limited to a single season's data collection which coincided with pruning in a field that was going into an "on" year

4. Further explore leaf nutrients and carbohydrate analysis relationships with yield. Correlations found between nutrient in the leaves and subsequent yields in this research are promising but it is important to note that the data set obtained was limited (10 trees). A more comprehensive investigation into the nutrient levels and carbohydrates in leaves is recommended.

5. Investigate carbohydrates in younger trees. Study the response in carbohydrates by manipulating the trees using treatments such as leaf or flower removal.

REFERENCES

Acuña-Maldonado, LE, Smith, M, Maness, N, Cheary, B, Carroll, B & Johnson, G 2003, 'Influence of nitrogen application time on nitrogen absorption, partitioning, and yield of pecan', *American Society for Horticultural Science*, vol. 128, pp. 155-62.

AFIA Laboratory methods manual 2005, *Determination of water-soluble carbohydrates – water extraction – alkaline ferricyanide*, Method-1.11A.

Amling, HJ & Amling, KA 1984, 'Physiological differentiation of pistilalte flower of pecan and cold requirements for their initiation', *American Society for Horticultural Science*, vol. 108, no. 2, pp. 195-8.

Aune, D, Keum, N, Giovannucci, E, Fadnes, LT, Boffetta, P, Greenwood, DC, Tonstad, S, Vatten, LJ, Riboli, E & Norat, T 2016, 'Nut consumption and risk of cardiovascular disease, total cancer, all-cause and cause-specific mortality: a systematic review and dose-response meta-analysis of prospective studies', *BMC Medicine*, vol. 14, no. 1, p. 207.

Australian Nut Industry Council 2019, *Australias's tree nut industry*, Hort Innovation, Wangaratta, Victoria.

Bao, Y, Hu, FB, Giovannucci, EL, Wolpin, BM, Stampfer, MJ, Willett, WC & Fuchs, CS 2013, 'Nut consumption and risk of pancreatic cancer in women', *British Journal of Cancer* vol. 109, no. 11, pp. 2911-6.

Barwick, J 2011, 'Carbohydrate reserves to help predict yields', *The Australian & New Zealand Grapegrower & Winemaker*, no. 568, pp. 28-9.

Beuchat, L & Mann, D 2010, 'Survival and growth of salmonella in high-moisture pecan nutmeats, in-shell pecans, inedible nut components, and orchard soil', *Journal of Food Protection*, vol. 73, pp. 1975-85.

Briceño Contreras, E, Valenzuela-Núñez, L, Garcia-De La Peña, C, Esparza-Rivera, JR & Borja-De la Rosa, A 2018, 'Contenido de almidón en órganos de nogal (*Carya illinoensis Koch*) en dos etapas fenológicas', *Revista Mexicana de Ciencias Agricolas*, vol. 20, pp. 4161-73.

Briceño Contreras, E, Moreno-Reséndez, A, Valenzuela-Núñez, L, Garcia-De La Peña, C, Esparza-Rivera, J & Rodríguez-Martinez, R 2019, 'Influence of temperature and irradiation on starch concentration in *carya illinoinensis K. Koch* varieties wichita and western', *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, vol. 25, pp. 305-14.

Brison, FR 1974, *Pecan culture*, Capital Print, Austin, Texas, U.S.A.

Bureau of Meteorology 2021, *Daily rainfall Pallamallawa*, viewed 27 March 2021, <http://www.bom.gov.au/jsp/ncc/cdio/weatherData/av?p_nccObsCode=136&p_display_type=dailyDataFile&p_startYear=&p_c=&p_stn_num=053033>.

Bustan, A, Avni, A, Lavee, S, Zipori, I, Yeselson, Y, Schaffer, AA, Riov, J & Dag, A 2011, 'Role of carbohydrate reserves in yield production of intensively cultivated oil olive (*Olea europaea L.*) trees', *Tree Physiology*, vol. 31, no. 5, pp. 519-30.

Byford, R 2005, *Growth and development of pecan nuts*, Department Head, Extension Plant Sciences, New Mexico.

Cameron, SH 1923, 'Storage of starch in the pear and apricot', *American Society for Horticultural Science*, vol. 20, pp. 98-100.

Chapin, F, Schulze, E & Mooney, H 1990, 'The ecology and economics of storage in plants', *Annual Review of Ecology, Evolution, and Systematics*, vol. 21, pp. 423-47.

Cheng, L & Robinson, T 2004, 'Management of nitrogen and carbohydrate reserves to improve growth and yield of apple trees', *Fruit Quarterly*, 01/01, vol. 12, no. 3, pp. 19-22.

Cheng, L, Ma, F & Ranwala, D 2004, 'Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply', *Tree Physiology*, vol. 24, pp. 91-8.

Conner, PJ & Worley, RE 2000, 'Alternate bearing intensity of pecan cultivars', *Journal of the American Society for Horticultural Science*, vol. 35, pp. 1067-9.

Crane, HL, Hardy, MB, Loomis, NH & Dodge, FN 1934, 'Effect of nut thinning on size, degree of filling, and annual yields of pecans', *American Society for Horticultural Science*, vol. 45, pp. 59-62.

Crane, JC & Al-Shalan, I 1997, 'Carbohydrate and nitrogen level in pistachios as related to shoot extension and yield ', *American Society for Horticultural Science*, vol. 102, no. 4, pp. 396-9.

Da Silva, D, Qin, L, Debuse, C & Dejong, T 2014, 'Measuring and modelling seasonal patterns of carbohydrate storage and mobilization in the trunks and root crowns of peach trees', *Annals of Botany* vol. 114, pp. 643-52.

Davis, JT & Sparks, D 1974, 'Assimilation and translocation patterns of carbon-14 [radioactive carbon] in the shoot of fruiting pecan trees, *Carya illinoensis Koch.*', *American Society for Horticultural Science*, vol. 99, no. 5, pp. 468-80.

Fisher, H 2009, *Grapevine physiology- a primer for serious wine growers*, Premium Production Practices Field Day, DoP Agriculture/Vineland, University of Guelph, Guelph, Ontario.

Furze, M, Huggett, B, Aubrecht, D, Stolz, C, Carbone, M & Richardson, A 2018, 'Whole-tree nonstructural carbohydrate storage and seasonal dynamics in five temperate species', *New Phytologist*, vol. 221, pp. 1466-77.

Glerum, C 1980, 'Food sinks and food reserves of trees in temperate climates', *New Zealand Journal of Forestry Science*, vol. 10, no. 1, pp. 176-85.

Goldschmidt, E 1999, 'Carbohydrate supply as a critical factor for citrus fruit development and productivity', *Journal of the American Society for Horticultural Science*, vol. 34, no. 6, pp. 1020-4.

Google Earth 2015, *Australia map*, Google Earth

Graham, C 2019, 'Understanding pecan tree dichogamy', *Pecan South* vol. 52, no. 10, pp. 14-25.

Gulati, S & Misra, A 2017, 'Effect of almond supplementation on glycemia and cardiovascular risk factors in asian indians in North India with type 2 diabetes mellitus: a 24-week study', *Metabolic syndrome and related disorders*, vol. 15, pp. 98-105.

Haddad, E, Jambazian, P, Karunia, M, Tanzman, J & Sabaté, J 2006, 'A pecan-enriched diet increases γ-tocopherol/cholesterol and decreases thiobarbituric acid reactive substances in plasma of adults', *Nutrition Research* vol. 26, pp. 397-402.

Hadgraft, B 2019, 'A cracking good idea', *The Farmer* pp. 32-8.

Hagidimitriou, M & Roper, T 1994, 'Seasonal changes in nonstructural carbohydrates in cranberry', *American Society for Horticultural Science* vol. 119, pp. 1029-33.

Heerema, R 2006, 'Alternate bearing research: implications for western pecans ', *Western Pecan Growers Association*, New Mexico, p. 7.

Heerema, RJ, Wells, ML, Lombardini, L, Nesbitt, M, Warren, C, Pegg, RB & Gong, Y 2016, 'Pecan kernel antioxidant capacity and oil composition are affected by mechanical pruning and by nut position in tree canopy', *Acta Horticulturae*, vol. 145, no. 3, pp. 499-506.

Herrera, E 1998, 'Carbohydrate storage and utilization', *Pecan South*, June, vol. 31, no. 5, p. 10.

Hopkins, WG & Huner, NPA 2008, *Introduction to plant physiology*, The University of Western Ontario, London, Ontario.

Iglesias, D, Tadeo, F, Primo-Millo, E & Talon, M 2003, 'Fruit set dependence on carbohydrate availability in citrus trees', *Tree Physiology*, vol. 23, pp. 199-204.

International Nut & Dried Fruit 2017, *Nuts & Dried Fruits Statistical Yearbook 2016/2017*.

International Nut & Dried Fruit 2018, *Nuts & Dried Fruits Statistical Yearbook 2017/2018*.

International Nut & Dried Fruit 2019, *Nuts & dried fruis statistical yearbook 20018/2019*.

Khan, ZU, McNeil, DL & Samad, A 1998, 'Root pruning of apple trees grown at ultra-high density affects carbohydrate reserves distribution in vegetative and reproductive growth', *New Zealand Journal of Crop and Horticultural Science*, vol. 26, no. 4, pp. 291-7.

Kim, T & Wetzstein, H 2005, 'Seasonal fluctuations in nutrients and carbohydrates in pecan leaves and stems', *The Journal of Horticultural Science and Biotechnology*, vol. 80, no. 6, pp. 681-8.

Kozlowski, TT 1992, 'Carbohydrate sources and sinks in woody plants', *The Botanical Review*, vol. 58, no. 2, pp. 107-222.

Kraimer, RA, Lindemann, WC & Herrera, EA 2001, 'Distribution of 15N-labeled fertilizer applied to pecan: a case study', *American Society for Horticultural Science*, vol. 36, no. 2, pp. 308-12.

Kraimer, RA, Lindemann, WC & Herrera, EA 2004, 'Recovery of late-season 15N-labeled fertilizer applied to pecan', *American Society for Horticultural Science*, vol. 39, no. 2, pp. 256-60.

Krezdorn , AH 1955, 'The nutrient status of pecan leaves in relation to alternate bearing', A& M College of Texas, Texas.

Liu, X, Robinson, P, Madore, M, Witney, G & Arpaia, M 1999, 'Hass' avocado carbohydrate fluctuations. I. Growth and phenology', *Journal of the American Society for Horticultural Science*, vol. 124, pp. 671-5.

Loescher, WH 1990, 'Carbohydrate reserves, translocation, and storage in woody plant roots', *American Society for Horticultural Science*, vol. 25, pp. 274-81.

Malstrom, HL 1974, 'The relationship of stored reserves to yield in mature pecan trees', *Western Pecan Conference*, New Mexico, pp. 65-6.

Martinez Diaz, G, Sabori Palma, R & Nunez Moreno, HH 2012, 'Carbohidratos en raices del nogal pecanero (*Carya illinoinensis Koch*) y su relacion con la alternancia en la production', *XIII Simposio Internacional de Nogal Pecanaero* Sonora, Mexico, pp. 80-4.

McEachern, GR 2013, 'Many pecans questions remain', *Pecan South* vol. 46, no. 4, p. nd.

McEachern, GR 2018, 'Uncovering the mystery of pecan pistillate flowering', *Pecan South*, vol. 51, no. 2, pp. 48-51.

McEachern, GR 2019, 'The course of alternative bearing', *Pecan South Magazine*, vol. 52, no. 9, pp. 18-24.

McKay, LD, Eliasziw, M, Chen, YOC & Blumberg, BJ 2018, 'A pecan-rich diet improves cardiometabolic risk factors in overweight and obese adults: a randomized controlled trial', *Nutrients*, vol. 10, no. 3, p. 339.

McQueen, JC, Minchin, PEH & Silvester, WB 2004, 'Changes in non-structural carbohydrate concentration in 1-year-old shoots of 'Braeburn' apple *(Malus domestica)* over two consecutive years', *New Zealand Journal of Crop and Horticultural Science*, vol. 32, no. 3, pp. 319-23.

Meyer, R 2020, *Carbohydrates methodology*, 8 July 2020.

Monselise, SP & Goldschmidt, EE 1982, 'Alternate bearing in fruit trees', *Horticultural Reviews*, vol. 4, pp. 128-73.

NSW Department of Primary Industries n.d, *Why phosphorous is important*, viewed 11 Aug 2021, <https://www.dpi.nsw.gov.au/agriculture/soils/improvement/phosphorous>.

Nzima, MDS, Martin, GC & Nishijima, C 1997, 'Seasonal changes in total nonstructural carbohydrates within branches and roots of naturally "off" and "on" `kerman' pistachio trees', *Journal of the American Society for Horticultural Science* vol. 122, no. 6, pp. 856-62.

Oliveira, C & Priestley, C 1988, 'Carbohydrate reserves in deciduous fruit trees', in *Horticultural Reviews* vol. 10, pp. 403-30.

Pallardy, S 2008, *Physiology of Woody Plants*, 3rd edn, Missouri.

Pearce, SC & Dobersek-Urbanc, S 1967, 'The measurement of irregularity in growth and cropping', *American Society for Horticultural Science*, vol. 42, pp. 295-305.

Rajaram, S, Burke, K, Connell, B, Myint, T & Sabaté, J 2001, 'A monounsaturated fatty acid–rich pecan-enriched diet favorably Aaters the serum lipid profile of healthy men and women', *The Journal of Nutrition*, vol. 131, no. 9, pp. 2275-9.

Regier, N, Streb, S, C Zeeman, S & Frey, B 2010, 'Seasonal changes in starch and sugar content of poplar (*Populus deltoides × nigra cv. Dorskamp*) and the impact of stem girdling on carbohydrate allocation to roots', *Tree Physiology* vol. 30, pp. 979-87.

Reibel, D 2019, *Farm information*, personal communication, November 2019.

Rice, GW 1994, *A grower's perspective: pecans*, PecanQuest Publications, Kansas.

Rohla, C, Smith, M, Maness, N & Reid, W 2007, 'A comparison of return bloom and nonstructural carbohydrates, nitrogen, and potassium concentrations in moderate and severe alternate-bearing pecan cultivars', *Journal of the American Society for Horticultural Science*, vol. 132, pp. 172-7.

Rohla, cT 2013, *Research updates pecan tree fertilizer recommendations*, Noble Research Institure, viewed 17 May 2022, <https://www.noble.org/news/publications/ag-news-and-views/2013/march/research-updates-pecan-tree-fertilizer-recommendations/#:~:text=Phosphorus%20(%20P%20)%20reduces%20leaf%20scorch,varieties%20(Smith%2C%202012).>.

Sala, A, Woodruff, D & Meinzer, F 2012, 'Carbon dynamics in trees: Feast or famine?', *Tree Physiology*, vol. 32, pp. 764-75.

Simpsoms, J 2016, *Pecan industry expansion*, PrimeFact.

Smith, AM & Stitt, M 2007, 'Coordination of carbon supply and plant growth', *Plant, Cell and Environment*, vol. 30, no. 9, pp. 1126-49.

Smith, CL & Waugh, JG 1938, 'Seasonal variations in the carbohydrate and nitrogen content of roots of bearing pecan tree', *Agricultural Research*, vol. 57, no. 6, pp. 449-60.

Smith, M 2005, 'Understanding alternate bearing', *Pecan South*, vol. 38, no. 10, pp. 32-7.

Smith, M, Rohla, C & Maness, N 2007, 'Correlations of crop load and return bloom with root and shoot concentrations of potassium, nitrogen, and nonstructural carbohydrates in pecan', *Journal of the American Society for Horticultural Science*, vol. 132, pp. 44-51.

Smith, MW, McNew, RW, Ager, PL & Cotten, BC 1986, 'Seasonal changes in the carbohydrate concentration in pecan shoots and their relationship to flowering', *American Society for Horticultural Science*, vol. 111, no. 4, pp. 558-61.

Sparks, D 1992, *Pecan cultivars, the orchard's foundation*, Pecan Production Innovations, Georgia.

Sparks, D 1996, 'A climatic model for pecan production under humid conditions', *American Society for Horticultural Science*, vol. 121, no. 5, pp. 908-14.

Sparks, D 1997, 'A model for predicting pecan production under arid conditions at high elevations', *American Society for Horticultural Science*, vol. 122, no. 5, pp. 648-52.

Sparks, D 2005, 'Adaptability of pecan as a species', *American Society for Horticultural Science*, vol. 40, pp. 1175-89.

Stafne, ET, Melanson, RA & Wilson, J 2017, *Fertilizing pecan trees* Mississipi State University, Mississipi

Stephenson, RA, Gallagher, EC & Rasmussen, TS 1989, 'Effects of growth manipulation on carbohydrate reserves of macadamia trees', *Scientia Horticulturae*, vol. 40, no. 3, pp. 227-35.

Thompson, MY, Randall, J, Heerema, R, J. & VanLeeuwen, D 2019, 'Exogenous plant growth regulators show promise for management of alternate bearing in pecan', *American Society for Horticultural Science*, vol. 54, no. 7, pp. 1204-7.

Tromp, J 1983, 'Nutrient reserves in roots of fruit trees, in particular carbohydrates and nitrogen', *Plant and Soil*, vol. 71, no. 1, pp. 401-13.

Valenzuela-Núñez, L, Briceño Contreras, E, Esparza-Rivera, J, Garcia-De La Peña, C, Rodríguez-Bautista, G & Núñez-Colima, J 2019, 'Seasonal changes in soluble sugar

concentration in pecan perennial organs (*Carya illinoiensis (Wangenh) Koch*)', *Acta Universitaria* vol. 29, pp. 1-13.

Vimpany, I 2016, *Total non-structural carbohydrate testing in macadamias*, Horticulture Innovation Australia, Sydney.

Vreugdenhil, D 1985, 'Source-to-sink gradient of potassium in the phloem', *Planta*, vol. 163, no. 2, pp. 238-40.

Wang, A-Y, Han, S-J, Zhang, J-H, Wang, M, Yin, X-H, Fang, L-D, Yang, D & Hao, G-Y 2018, 'The interaction between nonstructural carbohydrate reserves and xylem hydraulics in Korean pine trees across an altitudinal gradient', *Tree Physiology*, vol. 38, no. 12, pp. 1792-804.

Weckler, P, Jones, C, Bowser, T, Hardin, J, Wang, N, Franzen, A, Maness, N, Smith, M & Mathanker, S 2015, 'Technologies for enhancing pecan production and processing', *Acta Horticulturae*, vol. 1070, pp. 235-43.

Wells, L 2017, *Pecan: America's native nut tree*, Alabama.

Wells, L 2018, 'Mechanical hedge pruning affects nut size, nut quality, wind damage, and stem water potential of pecan in humid conditions', *American Society for Horticultural Science*, vol. 53, no. 8, pp. 1203-7.

Wells, L 2021, 'late-season water demand: how much is enough?', *Pecan South*, vol. 54, no. 6, pp. 6-11.

Wells, L & Conner, P 2007, *Southeastern pecan growers' handbook*, Cooperative Extension Service, University of Georgia College of Agricultural & Environmental Sciences.

Whiley, AW, Rasmussen, TS, Saranah, JB & Wolstenholme, BN 1996, 'Delayed harvest effects on yield, fruit size and starch cycling in avocado (*Persea americana Mill.*) in subtropical environments. I. The early- maturing cv. Fuerte', *Scientia Horticulturae*, vol. 66, pp. 23-34.

Wilkinson, J 2005, 'Pecans', in *Nut grower's guide: the complete handbook for producers*, Landlinks Press, Victoria, Australia, ch 14, pp. 157-73.

Wolstenholme, BN & Whiley, AW 1989, *Carbohydrate and phenological cycling as management tools for avocado orchards* South African Avocado Growers' Association Yearbook, Natal, Pietermaritzburg.

Wolstenholme, BN & Whiley, AW 1997, *What do carbohydrate reserves tell us about avocado orchard management?*, South African Avocado Grower's Association Yearbook, Natal, Pietermaritzburg

Wood, BW 1990, 'Alternate bearing of pecan ', *Pecan Husbandry: Challenges and Opportunities*, Unicor State Park, Georgia, pp. 180-7.

Wood, BW 1995, 'Relationship of reproductive and vegetative characteristics of pecan to previous-season fruit development and postripening foliation period', *Journal of the American Society for Horticultural Science*, vol. 120, no. 4, pp. 635-42.

Wood, BW 2011, 'Influence of plant bioregulators on pecan flowering and implications for regulation of pistillate flower initiation', *Journal of the American Society for Horticultural Science*, vol. 46, pp. 870-7.

Wood, BW 2012, 'Regulation of flowering in pecan', *Western Pecan Growers Association Conference* Las Cruces, New Mexico, pp. 20-3.

Wood, BW 2014, '"Late winter/early Spring" xylem sap characteristics influence pecan crop load', *American Society for Horticultural Science*, vol. 49, no. 7, pp. 886-90.

Wood, BW & McMeans, JL 1981, 'Carbohydrate changes in various organs of bearing and non bearing pecan trees', *American Society for Horticultural Science*, vol. 106, no. 6, pp. 758-61.

Wood, BW & Reilly, CC 2001, 'Atypical Symptoms of Cold Damage to Pecan', *American Society for Horticultural Science*, vol. 36, no. 2, pp. 298-301.

Wood, BW, Payne, JA & Grauke, LJ 1994, 'An overview of the evolution of the U.S pecan industry', in CR Santerre (ed.), *Pecan Technology*, Chapman & Hall New York, pp. 1-11.

Wood, BW, Conner, PJ & Worley, RE 2003, 'Relationship of alternate bearing intensity in pecan to fruit and canopy characteristics', *Journal of the American Society for Horticultural Science*, vol. 38, pp. 361-6.

Wood, BW, Conner, PJ & Worley, RE 2004, 'Insights into alternate bearing of pecan', *Acta Horticulturae*, vol. 636, pp. 617-29.

Wood, BW, Wells, L & Funderburke, F 2010, 'Influence of elevating tree potassium on fruit drop and yield of pecan', *American Society for Horticultural Science*, vol. 45, no. 6, pp. 911-7.

Wood, RA 1970, *A study of the characteristics of the pecan tree for use in yield forecast*, Research and Development Branch Standards and Research Division United States.

Worley, RE 1979a, 'Fall defoliation date and seasonal carbohydrate concentration of pecan wood tissues ', *American Society for Horticultural Science*, vol. 104, pp. 195-9.

Worley, RE 1979b, 'Pecan yield, nutlet set, and spring growth as a response to time of fall defoliation', *American Society for Horticultural Science*, vol. 104, no. 2, pp. 192-4.

Worley, RE 1994a, 'Pecan production', in CR Santerre (ed.), *Pecan technology*, Chapman & Hall, New York, ch 2, pp. 12-38.

Worley, RE 1994b, 'Pecan physiology and composition', in CR Santerre (ed.), *Pecan technology*, Chapman & Hall, Inc, New York, ch 3, pp. 39-48.

Wright, GC, Storey, JB, Harris, MK & Sprinz, PT 1990, 'Pre-harvest pecan yield estimation', *American Society for Horticultural Science*, vol. 25, no. 6, pp. 698-700.

APPENDIX I: TREE SAMPLING LAYOUT

Table 0.1: Selected trees layout for field A1

Block	A1		Irrigation	Drip
Variety	Wester Schley		**Row number orientation**	West-East

Layout	**A1**				
Row Number	**37**	**36**	**35**	**34**	**33**
Row Variety	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**
Tree position within row					
1	X		X		X
2	X		X		X
3	X		45		1
4	X		44		2
5	X		43		3
6	X		X		4
7	46		42		X
8	47		41		5
9	48		X		6
10	X		40		7
11	49		39		8
12	X		X		X
13	50		X		9
14			38		10
15			X		11
16			X		12
17			37		13
18			36		14
19			35		15
20			34		16
21			33		X
22			X		X
23			32		17
24			31		18
25			30		19
26			X		X
27			X		20
28			29		21
29			28		22
30			X		23
31			X		24
32			X		X
33			27		25

	34			26			

X represents trees skipped due to non-uniformity

Table 0.2: Selected trees layout for field C2

Block	C2
Variety	Wichita

Irrigation	Drip
Row Orientation	South-North

Layout	C2						
Row Number	**26**	**25**	**24**	**23**	**22**	**21**	**20**
Row Variety	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**
Tree position within row							
1	x		x		x		x
2	x		x		x		x
3	x		x		x		x
4	x		50		1		2
5	46		49		x		
6			48		3		
7			47		4		
8			x		5		
9			45		x		6
10			44		7		
11			43		8		
12			42		x		9
13			41		10		
14			40		11		
15			39		12		
16			38		13		
17			37		x		14
18			36		x		15
19			35		16		
20			34		17		
21			33		18		
22			32		19		
23			31		20		
24			30		21		
25			29		22		
26			28		23		
27			27		24		
28			26		x		
29			x		25		
30			X		23		
31			X		24		
32			X		X		
33			27		25		
34			26				

106

X represents trees skipped due to non-uniformity

Table 0.3: Selected trees layout for field C3

Block	C3		Irrigation	Drip
			Row number	South-
Variety	Western Schley		**orientation**	North

Layout	C3				
Row Number	**24**	**23**	**22**	**21**	**20**
Row Variety	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**
Tree position within row					
1	x	x	x	x	x
2	x	x	x	x	x
3	49		48		1
4	50		47		2
5			46		3
6			45		4
7			44		5
8			43		6
9			42		7
10			41		8
11			40		x
12			x		9
13			39		10
14			x		11
15			38		12
16			37		13
17			36		14
18			35		15
19			x		16
20			34		17
21			33		18
22			32		19
23			31		20
24			30		21
25			29		22
26			28		23
27			27		24
28			26		25
29					
30					
31					

32					
33					
34					

X represents trees skipped due to non-uniformity

Table 0.4: Selected trees layout for field D1

Block	D1		Irrigation	Flood
Variety	Western Schley		Row number orientation	South-North

Layout	D1						
Row Number	**30**	**29**	**28**	**27**	**26**	**25**	**24**
Row Variety	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**
Tree position within row							
1	x	x	x	x	x	x	x
2	x	x	x	x	x	x	x
3	41		40		1		
4	42		39		2		
5	43		38		3		
6	44		37		4		
7	45		36		5		
8	46		35		6		
9	47		34		7		
10	48		X		8		
11	49		32		9		
12	50		X		10		
13	33		30		11		
14	X		29		12		
15	X		28		13		
16	X		X		14		
17	27		26		15		
18	X		25		16		17
19	X		24		X		X
20	22		23		18		
21	X		X		19		
22	X		21		20		
23							
24							
25							
26							
27							
28							
29							
30							
31							
32							

33							
34							

X represents trees skipped due to non-uniformity

Table 0.5: Selected trees layout for field E4

Block	E4		Irrigation	Flood
			Row number orientation	South-North
Variety	Western Schley			

Layout	E4				
Row Number	**27**	**26**	**25**	**24**	**23**
Row Variety	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**
Tree position within row					
1	x	x		x	x
2	50	x	x	x	x
3			49		1
4			x		2
5			48		3
6			47		4
7			46		5
8			45		6
9			44		7
10			43		8
11			42		9
12			41		11
13			40		10
14			39		12
15			38		13
16			37		14
17			36		15
18			35		16
19			34		17
20			33		18
21			32		19
22			31		20
23			30		21
24			29		22
25			28		23
26			27		24
27			26		25
28					
29					
30					
31					
32					
33					
34					

X represents trees skipped due to non-uniformity

Table 0.6: Selected trees layout for field H4

Block	H4		Irrigation	Flood
Variety	Western Schley		Row number orientation	South- North

Layout	H4			
Row Number	**22**	**21**	**20**	**19**
Row Variety	Wichita	Western Schley	Wichita	Western Schley
Tree position within row				
1		x	x	x
2		x	x	x
3		x	x	x
4		x	x	x
5		x	x	x
6		x		1
7		50		2
8		49		3
9		48		4
10		47		5
11		46		6
12		45		7
13		44		8
14		43		9
15		42		10
16		41		x
17		40		11
18		39		12
19		38		13
20		37		14
21		36		15
22		35		16
23		34		17
24		33		18
25		32		19
26		31		20
27		30		21
28		29		22
29		28		23
30		27		24

	31		26		25
	32				
	33				
	34				

X represents trees skipped due to non-uniformity

Table 0.7: Selected trees layout for field H3

Block	H3
Variety	Wichita

Irrigation	Flood
Row number orientation	South-North

Layout	H3				
Row Number	**24**	**23**	**22**	**21**	**20**
Row Variety	**Wichita**	**Western Schley**	**Wichita**	**Western Schley**	**Wichita**
Tree position within row					
1	x		x		x
2	x		x		x
3	x		x		x
4	x		x		1
5	47		x		2
6	x		46		3
7	48		x		4
8	49		45		5
9	50		x		x
10			44		6
11			43		7
12			42		x
13			41		8
14			40		x
15			39		9
16			38		x
17			37		10
18			x		11
19			36		12
20			x		13
21			35		14
22			34		15
23			33		16
24			32		x
25			x		17
26			x		18
27			31		19
28			30		20
29			29		21

30			28		22
31			27		23
32			x		24
33			26		25
34					

X represents trees skipped due to non-uniformity

APPENDIX II: LABORATORY FORMS

Figure 0.1: Example submission form, NSW Department of Primary Industries, page 1

NSW **Department of Primary Industries**

Analysis Required

Standard Package (NIR) (Pasture, Hay, Forage, Silage, Straw)	Dry Matter, Ash Content, Total N & Crude Protein, NDF, ADF, Dry Matter Digestibility, Metabolisable Energy, WSC	☐
Premium Silage Package (NIR)	Standard Package + Silage pH and Silage Ammonia-N	☐
Healthy Horse Package (NIR)	Standard Package + Fat and Calc of DE for horses	☐
Grain/Concentrate Package (NIR)	Standard Package + Total Fat/Ether Extract	☐
Total Mixed Ration	Special calibration for mixed feed rations	☐
Wet Chemistry Feed Package	Standard Package + Total Fat/Ether Extract + WSC	☐
Wet Chemistry Forage Package	Standard Package + WSC (Wet Chemistry)	☐
Wet Chemistry By-Products Package	Standard Package + Total Fat/Ether Extract	☐

☐ Minerals[1] ☐ Starch * ☐ Bulk Density & Screenings*
☐ Chloride[3] ☐ Prussic Acid* ☐ Gross Energy*
☐ DCAD (incl Min & Chloride) ☐ Mould Count* ☐ Nitrate/Nitrite*
☐ Urea ☐ Full Mycotoxin scan[1] ☐ Energy (please tick):
☐ Pig DE* ☐ Fish DE* ☐ Poultry AME*

Other (please check website for options) (TNSC)

* Denotes tests not covered under the Laboratories NATA Scope of Accreditation

1 Denotes tests where service is subcontracted to an external provider. Preference is given to other NATA accredited Labs, and/or Govt facilities

PLEASE ASSIST RESEARCH BY COMPLETING THIS SECTION

What is the source of your sample ☑ Grown on Property ☐ Bought elsewhere

What will you use the feed for
☐ Beef Cattle ☐ Sheep
☐ Dairy Cattle ☐ Sale/Export
☐ Horse ☐ Other (please specify)

Standard or Silage Package
☐ Forage or Pasture ☐ Hay
☐ Silage ☐ Straw

☐ Maize ☐ Pasture-legume dominant ☐ Pasture-grass dominant
☐ Cereal ☐ Winter Forage Crop ☐ Summer Forage Crop
☐ Lucerne ☐ Other

Major Species   1 _____   2 _____

Stage of Growth:
☐ Early vegetative ☐ Late vegetative ☐ Boot
☐ Flowering ☐ Dough stage ☐ Seed development

Grain Concentrate Package
☐ Oats ☐ Wheat ☐ Barley
☐ Triticale ☐ Faba Beans ☐ Peas
☐ Lupins ☐ Maize ☐ Sorghum

Total Mixed Ration (TMR) / ByProducts Package (Wet Chemistry)
Primary Ingredients: 1. Pecan Bark   2. _____   3. _____   4. _____
Contains urea or other non-protein nitrogen (NPN) source: Yes ☐

Test results and findings may be provided to authorised staff and used for statistical, surveillance, extension, certification and regulatory purposes in accordance with Departmental policies. The information assists disease and residue control programs and underpins market access for agricultural products. The source of information will remain confidential unless otherwise required by Law or regulatory policies.

Figure 0.2: Example submission form, NSW Department of Primary Industries, page 2

LAB ID	**CLIENT ID**	**DESCIPTION**	**% TNSC**
R19-01347/0001	190601	Forage	13.9
R19-01347/0002	190603	Forage	12.8
R19-01347/0003	190605	Forage	17.6
R19-01347/0004	190606	Forage	11.8
R19-01347/0005	190608	Forage	12.3
R19-01347/0006	190612	Forage	14.4
R19-01347/0007	190618	Forage	12.3
R19-01347/0008	190619	Forage	17.7
R19-01347/0009	190620	Forage	11.9
R19-01347/0010	190623	Forage	12.7
R19-01347/0011	190625	Forage	18.2
R19-01347/0012	190626	Forage	12.3

Figure 0.3: Example results provided by NSW Department of Primary Industries

APPENDIX III: SUPPLEMENTARY RESULTS

Table 0.1: Mean TNSC (%) by month from August 2014 to July 2020 for the varieties studied Western Schley and Wichita and P values to assess the significance between varieties

Month	TNSC (%) mean P value		P values
	Western Schley	Wichita	
Jan	8.4992	12.3973	< 0.001
Feb	9.7842	14.7388	< 0.001
Mar	9.9045	14.8000	< 0.001
Apr	11.3636	16.3200	< 0.001
May	12.9870	17.7900	< 0.001
Jun	12.4091	17.1400	< 0.001
Jul	12.9000	17.3700	< 0.001
Aug	11.8829	16.2392	< 0.001
Sep	10.5354	15.0349	< 0.001
Oct	8.9738	12.8328	< 0.001
Nov	7.5643	12.6887	< 0.001
Dec	7.7824	12.7864	< 0.001

Table 0.2: Supplementary results from monthly TNSC (%) comparisons between "on" and "off" years. Averaged results from samples taken from August 2014 to April 2020

Independent Samples Test

Variety				Levene's Test for		t-test for Equality of Means					95% Confidence	
				F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Wester Schley	Jan	TNSC (%)	Equal variances assumed	0.728	0.403	0.831	21	0.415	0.749	0.900	-1.124	2.621
			Equal variances not assumed			0.891	17.407	0.385	0.749	0.841	-1.022	2.519
	Feb	TNSC (%)	Equal variances assumed	6.485	0.019	2.069	21	0.051	1.574	0.761	-0.008	3.157
			Equal variances not assumed			2.480	20.994	0.022	1.574	0.635	0.254	2.894
	Mar	TNSC (%)	Equal variances assumed	0.020	0.888	2.509	20	0.021	1.663	0.663	0.280	3.045
			Equal variances not assumed			2.619	16.658	0.018	1.663	0.635	0.321	3.004
	Apr	TNSC (%)	Equal variances assumed	3.527	0.080	-0.264	15	0.795	-0.186	0.703	-1.684	1.312
			Equal variances not assumed			-0.287	14.883	0.778	-0.186	0.647	-1.566	1.194
	May	TNSC (%)	Equal variances assumed	2.700	0.120	-1.387	16	0.185	-0.905	0.653	-2.289	0.478
			Equal variances not assumed			-1.230	8.559	0.252	-0.905	0.736	-2.584	0.773
	Jun	TNSC (%)	Equal variances assumed	2.945	0.107	1.833	15	0.087	1.389	0.757	-0.226	3.003
			Equal variances not assumed			2.062	13.659	0.059	1.389	0.673	-0.059	2.836
	Jul	TNSC (%)	Equal variances assumed	0.055	0.817	-0.510	16	0.617	-0.422	0.828	-2.176	1.332
			Equal variances not assumed			-0.515	13.352	0.615	-0.422	0.819	-2.187	1.343
	Aug	TNSC (%)	Equal variances assumed	0.110	0.743	0.074	26	0.941	0.030	0.402	-0.797	0.857
			Equal variances not assumed			0.073	21.546	0.943	0.030	0.412	-0.825	0.885
	Sep	TNSC (%)	Equal variances assumed	0.233	0.633	-2.528	25	0.018	-1.686	0.667	-3.059	-0.312
			Equal variances not assumed			-2.428	18.526	0.026	-1.686	0.694	-3.142	-0.230
	Oct	TNSC (%)	Equal variances assumed	0.071	0.793	-3.005	24	0.006	-2.462	0.819	-4.153	-0.771
			Equal variances not assumed			-3.083	23.383	0.005	-2.462	0.799	-4.112	-0.812
	Nov	TNSC (%)	Equal variances assumed	0.034	0.854	0.162	25	0.872	0.115	0.708	-1.343	1.572
			Equal variances not assumed			0.162	21.494	0.873	0.115	0.709	-1.358	1.588
	Dec	TNSC (%)	Equal variances assumed	1.413	0.246	1.569	25	0.129	1.073	0.684	-0.336	2.482
			Equal variances not assumed			1.641	24.426	0.114	1.073	0.654	-0.276	2.422
Wichita	Jan	TNSC (%)	Equal variances assumed	0.263	0.622	-1.113	8	0.298	-1.120	1.007	-3.442	1.201
			Equal variances not assumed			-1.039	5.121	0.346	-1.120	1.079	-3.874	1.633
	Feb	TNSC (%)	Equal variances assumed	0.354	0.568	-0.035	8	0.973	-0.055	1.588	-3.718	3.608
			Equal variances not assumed			-0.036	7.163	0.972	-0.055	1.546	-3.694	3.583
	Mar	TNSC (%)	Equal variances assumed	0.127	0.731	0.627	8	0.548	0.958	1.528	-2.566	4.482
			Equal variances not assumed			0.631	6.723	0.549	0.958	1.518	-2.662	4.578
	Apr	TNSC (%)	Equal variances assumed	1.441	0.275	-1.352	6	0.225	-0.767	0.567	-2.154	0.621
			Equal variances not assumed			-0.973	1.218	0.484	-0.767	0.788	-7.400	5.867
	May	TNSC (%)	Equal variances assumed	0.537	0.491	-3.877	6	0.008	-3.850	0.993	-6.280	-1.420
			Equal variances not assumed			-5.293	3.586	0.008	-3.850	0.727	-5.965	-1.735
	Jun	TNSC (%)	Equal variances assumed	1.675	0.243	3.699	6	0.010	3.033	0.820	1.027	5.040
			Equal variances not assumed			4.599	2.730	0.024	3.033	0.660	0.812	5.255
	Jul	TNSC (%)	Equal variances assumed	0.030	0.868	-0.993	6	0.359	-1.850	1.864	-6.410	2.710
			Equal variances not assumed			-0.957	1.646	0.458	-1.850	1.934	-12.147	8.447
	Aug	TNSC (%)	Equal variances assumed	0.106	0.753	-0.331	9	0.748	-0.306	0.926	-2.401	1.788
			Equal variances not assumed			-0.331	8.678	0.748	-0.306	0.925	-2.410	1.798
	Sep	TNSC (%)	Equal variances assumed	0.088	0.774	-1.721	8	0.124	-2.630	1.528	-6.154	0.895
			Equal variances not assumed			-1.721	7.720	0.125	-2.630	1.528	-6.177	0.917
	Oct	TNSC (%)	Equal variances assumed	0.414	0.535	-1.582	10	0.145	-1.855	1.172	-4.467	0.757
			Equal variances not assumed			-1.582	9.423	0.146	-1.855	1.172	-4.488	0.779
	Nov	TNSC (%)	Equal variances assumed	5.091	0.048	1.451	10	0.177	1.764	1.216	-0.944	4.473
			Equal variances not assumed			1.451	7.152	0.189	1.764	1.216	-1.098	4.626
	Dec	TNSC (%)	Equal variances assumed	0.010	0.923	0.849	10	0.416	1.227	1.446	-1.994	4.449
			Equal variances not assumed			0.849	9.855	0.416	1.227	1.446	-2.001	4.455