Characterizing Eastern Black Walnut (*Juglans nigra)* Cultivars for Alley Cropping Systems

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THESIS
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The undersigned, appointed by the Associate Vice Chancellor of the Office of Research and Graduate Studies, have examined the thesis entitled:

CHARACTERIZATION OF BLACK WALNUT (*JUGLANS NIGRA)* CULTIVARS FOR AGROFORESTRY SYSTEMS

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ABBREVIATIONS AND ACRONYMS

ACS	Alley Cropping Systems
ANOVA	Analysis of Variation
LCR	Live Crown Ratio
CrRt	Crown Retention
CrS	Trunk diameter
GHG	Greenhouse gas
HLC	Height to live crown
Ht	Tree height
IPCC	Intergovernmental Panel on Climate Change
jd	Julian date
LA	Leaf Area
LAI	Leaf Area Index
LER	Land Equivalency Ratio
LIDAR	Light Detection and Ranging
LLftA	Leaflet Area
LSD	Least Significant Difference
MAS	Marker-assisted Selection
NDVI	Normalized difference vegetation index
PAR	Photosynthetically -active radiation
PARt	PAR Transmittance
TauC	Cumulative Tau
TauI	Incidental Tau
TauP	Percentage Tau
UMCA	University of Missouri Center for Agroforestry
W	Canopy Width
RD	Ripe Date
SL	Season Length
AnthS	Anthracnose Susceptibility
GCA	General Combining Ability
SCA	Specific Combining Ability
LEE	Length end-to-end
WSS	Width suture-to-suture
WCC	Width cheek-to-cheek

THESIS ABSTRACT

The identification of complementary species combinations will accelerate the expansion of temperate agroforestry. Since the mid-19th century, European timber plantations have taken advantage of the late-leafing habit of walnut (*Juglans* spp.) to grow a spring grain crop between the tree rows. A parallel but underutilized opportunity in North American involves incorporating eastern black walnut (*Juglans nigra* L.) cultivars into alley cropping systems. Eastern black walnut is native to North America and exhibits architectural and phenological characters for reduced competition with winter alley crops. Black walnut also produces nutritious nuts, and cultivars with improved kernel percentage and mass offer potential to cultivate the species as a domesticated orchard crop, as opposed to just the high-quality timber for which it is well-known. However, field observations suggest significant variation in tree architecture and phenology amongst cultivars, which is likely to influence complementarity with winter grains. Comprehensive characterization of trait genetic diversity is needed to best leverage germplasm into productive systems~~.~~. First~~Here~~, ~~we review literature~~literature is reviewed related to implementing alley cropping systems (ACS) with consideration of cultivar-dependent traits that may reduce interspecific competition. ~~In addition~~In addition, an experiment was performed characterizing black walnut cultivars for their aboveground compatibility for light in~~with~~ alley cropping systems. A population of 11 cultivars with replicates in New Franklin, MO was measured for understory light infiltration from April-June of 2021. Results showed over a two-fold difference in light infiltration between the most contrasting cultivars during the second week of May. Tree architecture and phenological event timing were also measured to determine potential causative factors of differences observed. The impact of this research may help growers interested in walnut alley cropping with cultivar selection and management practices for effective agroforestry systems. While the compatibility between tree and crops is an important factor in agroforestry design, cultivars must also be commercially productive for robust adoption by growers. An additional study was performed on the performance of 7 selections from the UMCA Black Walnut Breeding Program. A replicated population of these selections were observed in 2017 and 2021 for key traits for improving the commercial production quality of black walnut such as yield, kernel percentage and kernel mass. Results showed selections were able to significantly outperform the parental generation in these traits by as much as 135%. Additional years of harvest data as well as replication sites for this trial will help refine the understanding of the commercial performance of selections and allow for broader recommendations to growers at large. While the focus in this thesis is directed toward black walnut, broad characterization of other underutilized fruit/nut species will allow for robust diversification of ACS.

CHAPTER 1: FRAMING THE UTILIZATION OF EASTERN BLACK WALNUT (JUGLANS NIGRA) CULTIVARS IN ALLEY CROPPING SYSTEMS

1.1 ABSTRACT

The identification of complementary species combinations will accelerate the expansion of temperate agroforestry. Since the mid-19th century, European timber plantations have taken advantage of the late-leafing habit of walnut (*Juglans* spp.) to grow a spring grain crop between the tree rows. Such alley cropping systems increase land-use efficiency and provide extensive environmental benefits. A parallel but underutilized opportunity in North American involves incorporating eastern black walnut (*Juglans nigra* L.) cultivars into alley cropping systems. Eastern black walnut is native to North America and exhibits architectural and phenological characters for reduced competition with winter alley crops. Black walnut also produces nutritious nuts, and cultivars with improved kernel percentage and mass offer potential to cultivate the species as a domesticated orchard crop, as opposed to just the high-quality timber for which it is well-known. However, field observations suggest significant variation in tree architecture and phenology amongst cultivars, which is likely to influence complementarity with winter grains. Comprehensive characterization of trait genetic diversity is needed to best leverage germplasm into productive systems. Here, we review literature related to implementing alley cropping systems (ACS) with consideration of cultivar-dependent traits that may reduce interspecific competition. While the focus is directed toward black walnut, broad characterization of other underutilized fruit/nut species will allow for robust diversification of ACS.

2.1 INTRODUCTION

Monoculture systems dominate the agricultural landscape of the Midwest US. While exceedingly productive, its environmental impacts are far-reaching. The use of frequent tillage practices on landscapes subject to wind erosion combined with minimal use of cover crops has caused nearly a one-third, or 30 million acres, loss in in carbon-rich topsoil across the midwestern Corn Belt (Thaler et al., 2021). As a direct result, there has been a reduction in crop yields equaling a $3 billion annual loss in farm income in this region. Additionally, top soil disturbance causes stored carbon to oxidize into the atmosphere, contributing to substantial greenhouse gas emissions (GHG) (Wang et al., 2020). In 2019, crop cultivation in the U.S. was responsible for an emitting and estimated 368mt of CO2-eq, an equivalent of an additional 80 million passenger vehicles on the road (EPA, 2022). As a result of elevated GHG in Earth's atmosphere, a 2021 report by the Intergovernmental Panel on Climate Change (IPCC) states that the rate of sea-level rise has tripled in the last 50 years. Temperatures are also predicted to rise above 1.5°C by the year 2040, causing an increase in the occurrence of extreme weather (Masson-Delmotte et al., 2021). Strategies to mitigate the environmental impact of monoculture cropping are critical moving forward.

Agroforestry practices improve the net impact of agricultural production (Pavlidis & Tsihrintzis, 2018). One such practice, alley cropping (i.e., growing crops between rows of trees), emphasizes using trees and shrubs as productive components of the farm, providing income from timber, fruits, nuts and multi-functional landscapes (Lovell et al., 2018; Mander et al., 2007). Trees and shrubs integrated into annual cropland reduce soil erosion, improve watershed health, and increase biodiversity (Bentrup, 2014; W. Stamps et al., 2009; Udawatta et al., 2002; Vacek et al., 2018; Wolz et al., 2018). The addition of woody biomass also aids in the sequestration of CO_2 as above and belowground terrestrial carbon pools. In less than ten years, an experimental ACS plot in Germany observed a more than doubling of soil organic carbon (Nii-Annang et al., 2009). A meta-analysis by DeStefano and Jacobson supports soil carbon sequestration by agroforestry systems, showing that soil organic carbon increased between 26-34% (depending on soil depth) when agricultural land was transitioned into agroforestry (2018). Udawatta and Jose

(2011) estimated that even modest adoption of alley cropping systems (ACS) and other tree-based agroforestry practices would offset annual US energy emissions by 34%. Furthermore, mixed-species ACS release up to 83% less nitrous oxide, an even more potent GHG, due to increased utilization of soil nitrogen (Wolz et al., 2018). While the climate mitigation and ecological arguments for integrating trees on cropland are strong, incentivized adoption will likely arise with increased on-farm productivity and profitability (Lovell et al., 2018).

Farmers need an economic rationale to adopt new strategies for managing their land and farming operations. Demonstrating alternative land-use practices that increase bottom line farm profitability or provide more stable revenue streams may help provide such a rationale. Evidence presented thus far suggests that increasing on-farm crop diversity can reduce profit volatility inherent in commodity agriculture (Brandes et al., 2016; Harkness et al., 2021). Specifically, uncorrelated revenue streams derived from multi-species ACS with grains, fruit, nuts and timber offer opportunity for financial stability by distributing risk and enhancing land-use efficiency (W. Stamps et al., 2009; Xu et al., 2019). Economic analyses of ACS on several sites in Missouri and Nebraska indicated profitable grain and legume yields planted between tree rows, in some cases with no significant difference in yield compared to the row crop monocultures (Godsey, 2003; W. Stamps et al., 2009). Economic and spatial analyses by Wolz and DeLucia (2019) supported these findings, determining that timber ACS in the midwestern US was a more favorable long-term investment than conventional corn production or forestry alone when targeting the appropriate environments. In addition, government incentives, such as the Environmental Quality Incentives Program offer farmers $50 per acre for alley cropping and more for socially disadvantaged farmers (Arango-Quiroga et al., 2018) although most payment contracts have terms of only 1-3 years.

Despite the potential of ACS in the Midwest, adoption has remained low. While farmers are interested in using agroforestry on their land (Mattia et al., 2018), scaling the adoption of ACS with specialty nut crops, such as Chinese chestnut *(Castanea mollissma* Blume) , hybrid hazelnut *(Corylus avellana L.* X *C. americana* Marshall) or eastern black walnut, will depend on the development of improved cultivars through breeding and selection (Coggeshall, 2011; Mori et al., 2017; Revord et al., 2019). A challenge to designing agroforestry systems is identifying compatible tree species and cultivars/breeding selections with desirable commercial traits **and** reduced interspecific competition with alley crops (Desclaux et al., 2016; Santi & Ferrandez, 2014).

This paper discusses the potential of an underutilized nut tree, eastern black walnut, to scale opportunity for ACS integration into the Midwest U.S. agricultural landscape. In doing so, we review the market and genetic improvements of eastern black walnut as a nut crop and detail cultivar-specific phenological traits relevant to ACS complementarity. This approach is the first step towards identifying and developing agroforestry-adapted cultivars of black walnut, which can be applied to many other tree crop species.

3. BLACK WALNUT FOR ALLEY CROPPING

3.1 *Juglans*, A Model Genus for ACS Diversification

Although the majority of global land currently utilizing ACS is found in the tropics, identifying instances of ACS in temperate regions can provide insights into how the practice may be implemented in North America (Wilson & Lovell, 2016). China has integrated fruit and nut trees onto croplands for centuries as a method of diversification and food security. Examples include Chinese chestnut with soybeans (*Glycine max* L. Merr.*)* in Yunnan Province, apricot (*Prunus armeniaca* L.) with wheat (*Triticale aestivum* L.) in the Northwest region, and apple (*Malus domestica* Borkh.) with soy and peanuts (*Arachis hypogaea* L.) in the Loess Plateau region (Gao et al., 2013; Qiao et al., 2020; Raj & Lal, 2014). The practice has since spread to Europe with winter cereal grain traditionally grown in alleys created between tree rows of sweet chestnut (*Castanea sativa* Mill.) in Galicia, Spain. In addition, rows of poplar (*Populus* spp.) and black locust (*Robinia pseudoacacia* L.) are successfully alley cropped with rye (*Secale cereale* L.) in the Lower Lusatia region of Germany (García Queijeiro, 1997; Nii-Annang et al., 2009).

While it is evident that temperate agroforestry utilizes many genera of trees, the most common is *Juglans*, which is studied in 34% of all field experiments (Wolz & DeLucia, 2018). This genus comprises wind-pollinated, monoecious hardwood trees in the *Juglandaceae* family (Zhu et al., 2019). Persian walnut (*J. regia*) is the most commercially successful agricultural species in the genus, growing on 440,000 acres in the US and producing a crop valued at nearly $1.3 billion annually (USDA, 2020). Persian walnut has been successfully alley cropped with tea (*Camellia sinensis* L. Kuntze) in the Yunnan Province of China, vegetables in Great Britain and (Leshem et al., 2009) and grapevines in Italy (Leshem et al., 2009; Nerlich et al., 2013; Paris et al., 2019). Unfortunately, commercial cultivation of Persian walnut in the US is restricted to states with warm mild climates found in California and surrounding states (Ebrahimi et al., 2017) and requires irrigation. Cold-tolerant *Juglans* species such as *J. cinera* (L.) and the Japanese walnut, *J. ailantifolia* (Carr.) and hybrids thereof also exist across the eastern North American landscape and produce edible nuts (Brennan et al., 2020; Pike et al., 2021). However, in comparison, black walnut is more broadly adaptable and disease resistant and thus presents a compelling option for expansion of North American temperate ACS.

3.2 A Nut Crop with Growing Potential for the Midwest U.S.

Eastern black walnut has extensive native range across the eastern US, extending from the Great Lakes Region to Florida and Texas (Salek & Hejcmanova, 2011). The quality of black walnut wood for timber and veneer is exceptional. It is the most valuable hardwood tree species in North America, with a standing stock valued at over a half-trillion dollars (Newton et al., 2009). Additionally, there is a regional market for the edible nuts of eastern black walnut, including uses in ice cream and baked goods (Wendholt Silva, 2016). An estimated 15.9 million kilograms of black walnuts are harvested annually to support products made from both their nuts and shells (Coggeshall, 2011; Wendholt Silva, 2016). Black walnuts have the highest protein content of commercially grown nuts and higher levels of many vitamins and minerals than the common Persian walnut (*J. regia*), the most economically important nut-tree species of the genus (Câmara & Schlegel, 2016). Current research also suggests that black walnut consumption may have protective effects against cardiovascular disease, neurodegeneration, diabetes and various cancers (Câmara & Schlegel, 2016; Vu et al., 2020). These effects are associated with anti-inflammatory metabolites such as flavonols, hydroxybenzoic acids, ellagitannins, and the species' unique fatty acid and vitamin profiles (Vu et al., 2020). Additionally, the nutshell is a high-value material used in industrial cleaning applications, oil well drilling, painting, and cosmetics industries (Michler et al., 2008).

The supply of black walnut nut meat for regional markets is primarily derived from harvesting wild, unimproved seedling trees (Coggeshall, 2011). After processing, the amount of edible kernel recovered from these nuts is only 10-15% of total nut weight, 4-8x less than the highly-domesticated Persian walnut (Reid, 1990). As a result, the leading processor of black walnut products, Hammons Products Company, limits their purchase price of wild nuts to around \$0.16 per pound (Hammons, 2016). An additional deterrent to the establishment of commercial plantings of black walnut is alternate bearing (i.e., the inter-annual yield variation). Nut quality attributes, including light kernel color and mild flavor, also vary across wild stands and harvesting practices, resulting in challenges to consistently meet consumer preferences (Reid et al., 2009). Cultivars noted for high nut production and kernel quality are available commercially. These trees tend to have a sprawling canopy with profuse flowering and fruiting along lateral and spur branches. This type of crown structure indicates high yields and is distinctly separate from the upright branchless attribute of timber cultivars (Reid et al., 2009). However, they are simply wild selections or chance seedlings (Zhao et al., 2018), not the products of organized breeding programs. While these currently available cultivars have enabled modest-sized first-generation orchards, there is great potential for their use in systematic breeding to develop new commercial releases that help standardize and scale the industry.

3.3 Walnut-Based ACS with Winter Crops

Black walnut has been utilized for timber in Europe since first introduced in the 17th century and is grown for timber on an estimated 20,000 ha across 14 European countries (Goodman et al., 2013; Nicolescu et al., 2017; Pelleri et al., 2020; Šálek & Hejcmanová, 2011). In addition to the high-quality wood, European growers note that black walnut trees better resist pests, diseases, and drought conditions than *J. regia* (Salek & Hejcmanova, 2011). However, even with good management practices, black walnut plantations have a minimum timber rotation period of 40 years (Nicolescu et al., 2017). To generate revenue in the interim, European farmers have alley-cropped wheat (*Triticum aestivum*, *T. durham* Desf.) and barley (*Hordeum vulgare*

L.) between the tree rows. The Dauphiné Province in southeastern France has been intercropping trees since antiquity as both timber and nut crops (Dupraz & Liagre, 2008). In this region, 1,500 ha of black walnut and hybrids (*J. nigra* X *regia)* are alley cropped, representing 80% of all walnut plantations under 10 years of age (Dupraz, 1994). Adopters of walnut-wheat ACS may have unknowingly buffered their grain crops against abiotic stressors. Recent modeling research of walnut-wheat ACS under various climate scenarios has predicted a reduction of heat, drought and nitrogen stressors up to 35% in the wheat crop (Reyes et al., 2021).

The integration of black walnut trees with winter crops can increase land-use efficiency and productivity, as measured by the land equivalency ratio (LER), a productivity ratio comparing the yield of mixed cropping systems with their component monocultures (Mead & Willey, 1980). An LER value higher than 1.0 indicates that the system's yield is greater than the sum of its parts. There have been several experiments examining the LER in walnut ACS with winter crops. Zhang (2015) showed Persian walnut-wheat systems in northwest China had significant yield advantages with an average LER of 1.45. Similar research conducted in western Spain reported LER as high as 2.08 and 1.72 in Persian walnut ACS with barley and winter wheat, respectively (Arenas-Corraliza et al., 2018). Exploration of tree physiology that lessens the competitive interactions with alley crops would offer direction for improving system productivity and diversifying ACS with other underutilized tree fruits and nuts.

3.4 Interspecific Competition in Black Walnut ACS

Resource partitioning in ACS occurs due to aboveground competition for light and belowground competition for water and nutrients. Belowground competition occurs when tree and alley crop roots utilize similar soil horizons for nutrient and water uptake (Zamora et al., 2008; Zamora et al., 2009). Such belowground competition can occur during key phenological stages (e.g., heading, grain filling), reducing alley crop yields (Fletcher et al., 2012; Jose et al., 2000; Zamora et al., 2009). Fortunately, when planted near row crops, black walnut's root architecture reduces competition by adapting to deeper soil profiles. (Andrianarisoa et al., 2015; Cardinael et al., 2015). Cultural practices can further reduce this root-zone overlap, such as root barriers, trenching, and annual "ripping" which may have positive effects on nut productivity in maturing orchards (Coggeshall, person comm.). However, despite managing belowground competition, crops growing directly adjacent to tree rows yielded less and grew to only 70% the height of those grown in the center of the alley (Miller & Pallardy, 2001; Zamora et al., 2008).

Multiple studies show that shade can reduce cereal crop yield up to 50% (Chirko et al., 1996; Dufour et al., 2013; Friday & Fownes, 2002; Li et al., 2008), and the magnitude of yield reduction depend on the intensity of shade and the physiological stage of the crop plant when the shade is introduced. Winter wheat and barley are planted in autumn, enter the flowering stage in spring, and are typically harvested in early summer. Artificial shading experiments on wheat crops have defined a critical period beginning 10-30 days before flowering. Heavy shade during this period can significantly decrease the number of grains per spike and overall grain weight at the final harvest. (Abbate et al., 1997; Artru et al., 2017; Dufour et al., 2013). However, if modest shade is provided, by a dormant tree canopy for instance, yields of wheat and barley can increase by 19% compared to full-sun treatments (Arenas-Corraliza et al., 2019). There may be additional critical periods not yet identified that are relevant to other potential alley crops harvested in the summer or fall.

Landscape-level design strategies to reduce competition for light in ACS are numerous. Tree rows can be spaced wider to decrease the amount of canopy shade. Naturally, black walnut

ACS with tree rows spaced 24 m apart produced greater alley crops than systems with rows spaced half as wide (McGraw et al., 2008; W. T. Stamps et al., 2009). This approach, however, can introduce high levels of spatial heterogeneity of crop irradiance, potentially resulting in uneven ripening and pre-harvest sprout damage (Dupraz et al., 2018; Vetch et al., 2019). Moreover, addressing aboveground competition with wider rows is undesirable, as it reduces the number of trees per acre and future income from the tree crop or timber harvest. Tree row orientation is also essential in maximizing light transmittance to crops in ACS and is a design decision that cannot be modified as after establishment. Modeling research on light competition shows that a North-South tree row orientation optimizes crop irradiation in hybrid walnut-wheat ACS located in latitudes greater than 50°. This is likely due to alley rows being closer to parallel with light beams originating from a south direction (or north direction if in the global south). In contrast, an East-West orientation is preferable in latitudes less than 40° latitude (Dupraz et al., 2018) where the tree rows would be closer to parallel with the sun's path in the sky, reducing shading onto the alley crops. However, in temperate zones between those latitudes, where much of the Midwest U.S. lies, tree phenology is a more significant factor of winter crop irradiation in ACS than row orientation (Dupraz et al., 2018). Thus, selecting tree species with delayed canopy formation can minimize aboveground competition for light. While not all tree crop species have black walnut's late-leafing habit, opportunities to improve and diversify temperate ACS will come from exploring tree phenology, physiology, and form to identify new species-specific traits that reduce competition for light.

4. APPLICATION AND ADVANCEMENT OF BLACK WALNUT ACS

4.1 Germplasm Characterization for Complementarity

Black walnut is one of the last temperate tree species to break dormancy in the spring and develop a full canopy (Mori et al., 2017). This behavior is thought to be an evolutionary adaptation to avoid spring frost damage to emerging buds and flowers, which occurs at temperatures below -3° C (Reid et al., 2009). Late bud break makes black walnut ideal for alley cropping with winter grains. Once its canopy is foliated, black walnut shows the most of amount of understory light infiltration compared to five common hardwoods (Mourelle et al., 2001). However, considerable genetic diversity for all spring phenological traits and tree architecture suggests thorough germplasm characterization will aid understanding of component traits that drive complementarity and how these traits vary amongst cultivars. For example, in central Missouri, bud break dates range over nearly 30 days within the species (Coggeshall, unpublished). Genetic variation for spring vegetative growth and canopy architecture is, however, not well characterized. Still, it appears diverse based on field observations, including traits like date of full crown closure, lateral branching behavior and overall canopy shape and size shape. Such characteristics contribute to photosynthetically active radiation (PAR) transmission through the canopy during the critical period for spring crops. Thus, teasing out their relative importance to transmissibility will offer insights to improving complementarity.

Germplasm availability is a major limitation to characterizing phenology and tree architecture. Collections that possess accessions/cultivars in replication and under relatively uniform conditions are required to draw comparisons amongst genotypes. Mature repositories, however, represent a long-term commitment of resources (i.e., funding, labor, space) and are thus quite rare. A repository of 70 black walnut cultivars was curated starting in 1996 at the Horticultural and Agroforestry Research Center in New Franklin, Missouri (39.01°N) to assemble specimens with high kernel percentage for replicated evaluation, conservation, and breeding (Coggeshall & Woeste, 2010). From 2002-2009, phenological characters (e.g., date of bud break, flowering, and harvest) were recorded (Coggeshall, unpublished), which sheds light on phenological variation relevant to alley cropping with winter wheat. Table 1.1 highlights the diversity in bud break amongst nut cultivars in the UMCA collection, which may reflect their diverse geographic origin.

Variation in bud break date should be compared with the key developmental stages of winter crops to gain insight into the potential interspecific interactions. Figure 1.1 displays the seasonal phenology of several black walnut nut cultivars and winter wheat, with the critical period for winter wheat enclosed in red. Bud break and harvest dates are based on historical data, while leafing and full canopy closure dates are estimated to depict these phases conceptually. More precise observation of the progression of vegetative development beginning at budbreak until full canopy closure would likely show additional variation to select individuals with greater complementarity for winter wheat.

Light transmittance through tree canopy can be influenced by multiple characters and varies by species (Mourelle et al., 2001); looking at bud break timing alone may not be sufficient. Morphological attributes such as total leaf area, tree dimensions and branching structure may all influence transmittance of photosynthetically-active radiation (PARt) in the understory (Talbot & Dupraz, 2011; Tang et al., 2019). To capture the suite of traits related to complementarity, characterization of cultivars throughout the critical period is required. Methodologies for physical measurements of isolated trees have been well established in forest management and tree physiology research (Fawcett et al., 2020; Grayson et al., 2012; Zhou et al., 2021). Modern techniques such as drone-acquired normalized vegetation index (NDVI) using multi-spectral sensors allow high-throughput and precise estimation of vegetative growth throughout the spring (Fawcett et al., 2020). Light detection and ranging (LIDAR) technology can accurately create 3D models of trees to analyze architectural parameters (Wang et al., 2008). While effective at tree biomass estimation, these approaches require expensive equipment and do not directly measure wavelengths of light utilized in photosynthesis. Field measurements of PARt using handheld devices such as the AccuPAR LP-80 present an inexpensive alternative to remote sensing and provide data relevant to crop growth in the understory.

4.2 Breeding Black Walnut Cultivars

Genetic improvement of black walnut is in its early stages. Although breeding has not yet pursued alley cropping complementarity, it is feasible for such objectives to be integrated into current goals of improving nut quality. In 2001, the University of Missouri Center for Agroforestry (UMCA) initiated a black walnut breeding program to improve the species for orchard nut production (Coggeshall, unpublished). The program began by assembling 84 black walnut cultivars identified on-farm or in the wild and named and propagated due to a high kernel percentage. Phenotypic data were collected on this collection between 2002 and 2013, demonstrating variation in economically important nut quality characters, phenological traits (Table 1.1), and resistance to walnut anthracnose (Coggeshall, 2011; McKenna & Coggeshall, 2018; Reid et al., 2009). Through both open and controlled pollinations, around 1500 progeny were produced from 2002 to 2008, comprising the program's first breeding generation. Progeny were evaluated from ages five to eight to identify outstanding individuals, namely for spur bearing, kernel percentage, mass, yield and low alternate bearing. Breeding selections from the

UMCA program have shown significant improvements in these commercial attributes compared to the previous generation of cultivars (Bishop et al., unpublished).

While most breeding goals of black walnut, and tree crops in general, have been related to increased crop quality and resistance to stressors, there is an opportunity to include compatibility with ACS as an additional consideration in selection. Improved tree cultivars, along with commensurate advances in shade-tolerance of alley crops, would aid in effective resource partitioning on agroforestry land. A black walnut breeding generation spans five years seed-to-seed, a relatively short generation time for temperate tree nuts. If seedlings can be phenotyped during this juvenile period for desirable traits, time delays common in tree breeding could largely be avoided and genetic gain accelerated. Moreover, as relevant alleles are identified, marker-assisted selection (MAS) and culling would be possible at year 1. Research on *J. regia* has identified and validated a marker for budbreak timing (Bernard et al., 2020), and quantitative trait loci (QTL) have been detected, explaining a portion of the variation in harvest date and several nut quality parameters (Aradhya et al., 2019). Similarly, research with the ‘Sparrow’ x ‘Shessler’ *J. nigra* mapping population at UMCA has identified QTL related to floral heterodichogamy and budbreak with future mapping targets of spur-bearing habit and kernel percentage.

5.1 FUTURE DIRECTIONS

The unique architectural and phenological traits of black walnut make it a compelling option for nut-based ACS. Characterization of cultivars for optimal complementarity will allow farmers to make informed decisions on the design of their agroforestry operations. To understand the mechanisms behind this complementarity, correlations between PARt, tree phenology, and morphology must be established. These associations will allow for rapid identification of ideal cultivars for ACS. Additionally, associations between tree architecture and PARt may guide spacings and pruning regimes for maximum light infiltration into the understory.

Exploration of the genetic basis of traits governing PARt may accelerate the development of cultivars with enhanced complementarity. The use of full-sibling experimental populations would allow for analysis of inheritance patterns as well as dominance and additive effects. Breeders could use this information to choose crosses with high likelihood of desirable traits in the progeny. In addition, if the black walnut mapping population reveals QTL for traits related to tree architecture and foliation patterns, as has been achieved in other hardwoods (Du et al., 2016; Socquet-Juglard et al., 2013), MAS of black walnut seedlings with both high yields and enhanced complementarity would be possible.

In addition to winter cereal grains, black walnut may be compatible with warm-season crops such as corn and soy, particularly during the juvenile stage of the trees. Black walnut cultivars with sparse summer canopies and defoliation in August, when corn and soybeans are ripening, may allow for reduced competition and ACS adoption across a much wider range of sites in the midwestern corn belt. Both of these crops, however, are sensitive to juglone which the black walnut secretes from its roots (Hejl & Koster, 2004; Jose & Gillespie, 1998), although may only be a concern in mid to late-stage systems. Research will be needed to design effective ACS with black walnut and warm-season crops that mitigate vectors of summer light displacement and juglone.

In addition to ACS, tree cultivars with high levels of spring PARt may be ideal for other agroforestry systems, particularly silvopasture environments, where light infiltration levels through tree canopy have been shown to affect forage quality and animal weight gain (Fannon et al., 2019). Late-leafing trees cast minimal shade on the pasture during spring while providing protection against heat and drought conditions to both pasture and livestock in summer (Beegle, 2019; Fike et al., 2017).

6.1 CONCLUSIONS

Increasing landscape diversity offers solutions to both ecological degradation and economic instability. Agroforestry practices provide an opportunity to integrate substantial populations of native productive tree species onto an otherwise homogenous environment. The potential of one such practice, walnut-wheat ACS in the Midwest US has been illustrated, emphasizing the importance of interspecific phenological complementarity. While ongoing research will identify optimal tree cultivars for use in such systems, farmers need not wait to integrate trees on their land as field grafting can occur many years after rootstock establishment.

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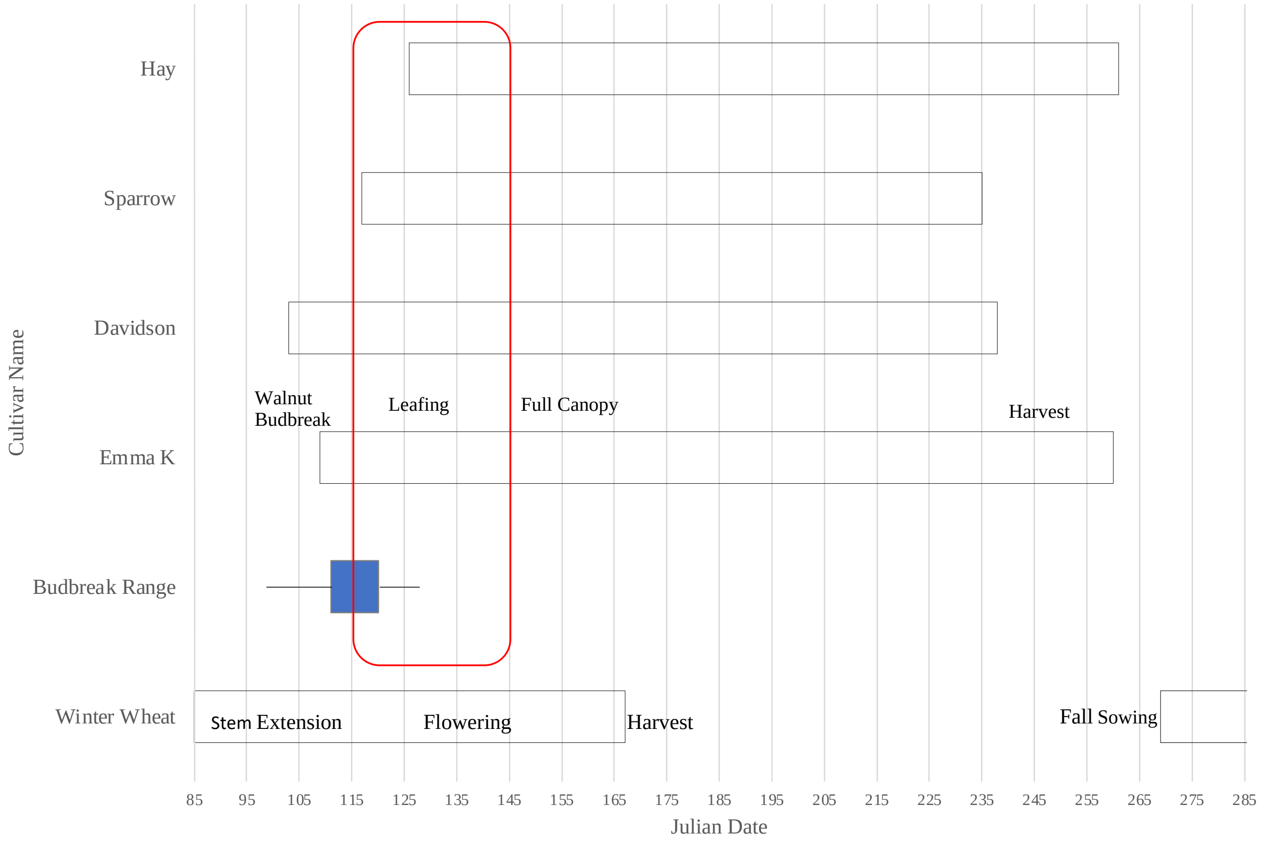
Table 1.1 Bud break dates of select black walnut cultivars. Date of bud break is recorded as days after ‘Shessler’, the earliest leafing cultivar, breaks bud. This occurs on April 8th on average. Confidence intervals at 95% are stated after bud break.

Cultivar	Bud break Date
Shessler	0 ± 1
South Fork	2 ± 1
Football	3 ± 1
Davidson	3 ± 1
McGinnis	4 ± 3
Jackson	4 ± 2
Krause	4 ± 1
Brown Nugget	4 ± 2
Emma K	6 ± 4
Rupert	6 ± 2
Tomboy	6 ± 2
Mystery	6 ± 3
Sparks 127	11 ± 5
Ohio	11 ± 6
Pound #2	12 ± 5
Kwik Krop	13 ± 4
Surprise	13 ± 6
Sparrow	14 ± 5
Daniel	15 ± 6
Bowser	17 ± 5
Hare	17 ± 5
Clermont	19 ± 6
Thomas	19 ± 6

Figure 1.1 The budbreak date of black walnut cultivars spans across the critical period (red box) where winter wheat is most sensitive to shade. The box plot in shows budbreak dates for the whole cultivar collection to demonstrate genetic variation and the opportunity to select for greater complementarity.



CHAPTER 2: CHARACTERIZING CANOPY LIGHT INFILTRATION IN BLACK WALNUT CULTIVARS

1.1 ABSTRACT

Eastern black walnut (*Juglans nigra* L.) is a nut-producing tree species that breaks dormancy late in the spring, allowing a winter alley crop to ripen before substantial canopy shade is formed. However, there is a high level of variation in the timing and structure of tree canopy development within the black walnut cultivar collection maintained at the University of Missouri Center for Agroforestry (UMCA). To identify cultivars with high levels of compatibility with winter alley cropping, we measured the amount of photosynthetically-active radiation transmitted (PARt) through the canopy. Eight physical traits were also measured to assess their relationship with light displacement and gain insight on characters that lead to great understory light infiltration. Significant differences between cultivars were seen in PARt, particularly on . Julian date 132 (May 12) where a two-fold difference was observed in PARt between the most contrasting cultivars. Traits such as date of bud break, tree shape and live canopy ratio were associated with levels of PARt, providing preliminary support for strong component drivers of light displacement/infiltration during spring. This study provides the first exploration of genetic variation in black walnut for above-ground compatibility with an understory crop, and in doing so, reports meaningful genetic variation exists for study and use. The data reported provide new context for design of subsequent genetic, physiological, plant-plant interaction studies. Additionally, these data may inform the selection or breeding of cultivars with crown ideotypes ideal for alley cropping with winter grains, as well as management strategies (pruning) to improve light infiltration black walnut alley cropping systems.

2.1 INTRODUCTION

The increasing global demand for food, timber and ecosystem services may be addressed by intensifying the photosynthetic capacity of arable land (Hussain et al., 2021). Towards this effort, strategies such as genetic improvement of staple crops and better use of fertilizers will be necessary but not sufficient to meet predicted requirements (Murchie et al., 2009). Novel agronomic practices that incorporate trees onto cropland, such as alley cropping systems (ACS), can support current approaches by bolstering net photosynthesis and productivity of the landscape (Forrester et al., 2013; He et al., 2013). The expanded use of ACS also offers environmental benefits through increasing ecosystem health and the sequestration of atmospheric carbon dioxide (Forrester et al., 2013; He et al., 2013; Pavlidis & Tsihrintzis, 2018; Wolz et al., 2018). However, light occlusion from tree leaves and branches limits productivity in understory crops (Artru et al., 2017; Gong et al., 2015; Li et al., 2016). Tree-crop combinations that effectively partition resources need to be utilized to address this concern. The phenological and architectural traits of walnut (*Juglans spp*.) complement winter cereal crops (Arenas-Corraliza et al., 2018; Dufour et al., 2013; Zhang et al., 2015), but there may be genetic variation within the species that allows for system improvement. Characterizing this diversity in eastern black walnut (*J. nigra* L.) presents an opportunity to identify tree cultivars ideal for North American agroforestry systems.

Multiple tree canopy traits can impact the amount of understory PARt (Mourelle et al., 2001). Bud break and canopy foliation are the most relevant phenological traits for complementarity in ACS and other mixed-species systems (Dupraz et al., 2018; West, 2014). Tree species with delayed phenological timing may offer reduced shading on winter grain crops while ripening in the spring (Artru et al., 2017; Dufour et al., 2013). In addition, tree architectural dimensions, crown shape, canopy volume and leaf morphology vary by species and affect PARt (Hagemeier & Leuschner, 2019; Mourelle et al., 2001; Sanusi et al., 2017). Mourelle (2001) found foliage biomass, the number of branches, LAI and crown width to be the best predictors for PARt within six temperate hardwood species. Leaf morphology and distribution in the canopy is also highly specific to species and appear to be related to the ecological role as early-succession species (e.g., *J. nigra* L.) or late-succession species (e.g. *Fagus grandifolia* Ehrh.) (Hagemeier & Leuschner, 2019; Mourelle et al., 2001).

Variation in phenological and morphological traits also occurs within an individual species, potentially due to contrasting selection pressures across geographies (Gailing et al., 2012; Sprinz et al., 1989). Leaf morphology was shown to vary significantly by cultivar in sweet chestnut (*Castanea sativa* Mill.) (Serdar & Kurt, 2011) and apple (Stephan et al., 2008). Canopy architecture also varied significantly by cultivar in apple (*Malus domestica* Borkh.) (Costes et al., 2003; Stephan et al., 2008), peach (*Prunus persica* L. Batsch*)* (Janick, 2010), olive (*Olea europea* L.) (Bayazit, 2012; Rallo et al., 2020) as well as in walnut species (Mosivand et al., 2013). Additional research on black walnut showed high levels of variation in canopy architecture, bud break and autumn crown retention (CrRt) within a population of 25 genotypes (Pang et al., 2021).

Characterizing cultivar-level variation in PARt, however, is largely unexplored. The diversity present within black walnut provides an opportunity to investigate how multiple component traits contribute to understory light infiltration throughout the spring. This research could inform both cultivar selection and management practices for effective ACS. It is anticipated that this approach may be replicated with other hardwood tree crop species such as Persian walnut (*Juglans regia*) and Chinese chestnut (*Castanea mollissima* Blume) to aid their integration onto cropland. Moreover, tree breeding programs can use characterized germplasm to inform crossing designs and selection criteria, representing the first step towards improving tree crops for complementarity in mixed species systems.

3. METHODS AND MATERIALS

3.1 Study Site

The study site is located at the Horticultural and Agroforestry Research Center in New Franklin, Missouri (39°01’05 N,92°45’42 W) and consists of two plots, Repository A and B, 0.9 hectares and 1.4 hectares respectively (Figure 2.1). Both repositories sit on deep well-draining deposits of loess derived menfro silt loam (5-15% slope), considered well-suited to black walnut cultivation (Wallace & Young, 2008). During tree establishment, a broad spectrum non-selective herbicide was applied in 1-meter strips along the tree lines. The plots were mowed throughout the growing seasons and were fertilized with 60 pounds of nitrogen in the spring and 40lbs in the fall.

3.2 Plant Materials

Black walnut trees are spaced 12m apart in an off-set grid pattern. In 1995-1997, trees were field-grafted into the repositories with named nut cultivars from various locations across the Midwest US. All ramets were genotyped by microsatellite SSR markers to confirm their correct identity and were relabeled if necessary (Zhao et al., 2018). From this collection, 11 cultivars (Table 2.1) were selected that displayed a broad range of phenological and architectural traits, including leafing date, season length, harvest date, canopy shape, and leaf morphology. For each cultivar, three ramets were selected randomly from within the repositories.

3.3 Phenology Data Collection

Every week beginning in mid-April 2021, trees were monitored for signs of bud break. They were classified according to the 5-stage scale, a method commonly used in hardwood tree phenology research (Gričar et al., 2017; Pang et al., 2021), where 1 is dormant and 5 is leaf expansion (Figure 2.2). Bud break was defined as date where 50% of the canopy had small leaves bursting from the bud (stage 4). On October 8, trees were visually evaluated for retention of foliage. The amount of foliation remaining on the tree was estimated to the nearest 10% and recorded as CrRt.

3.4 PAR Data Collection

PAR light infiltration readings with the LP-80 AccuPAR (Meter Environment) were taken in mid-April 2021 before the trees had broken dormancy, to establish a baseline. Thereafter, additional measurements were taken every 7-10 days during clear weather conditions between 11:00am and 2:00pm. One reading of open sky was taken immediately before a tree was measured to calibrate the device. In the understory of each tree, an inner-ring of 8 readings was taken adjacent to the trunk with the device aimed in each cardinal and intercardinal direction. An additional outer-ring of 8 readings was taken at the midpoint between the trunk and canopy dripline (Figure 2.3). Reading locations were marked on the ground for consistency. Measurements were collected on each tree until mid-June when canopy growth has ceased. AccuPAR divided the understory PARt readings by the corresponding open sky PAR reading to determine the percentage of light transmitted through the canopy into the understory, defined as Tau. Geographic location as well as time and date were recorded by the AccuPAR for estimation of solar zenith angle, beam fraction. Data stored in the AccuPAR were uploaded with LP-80 Utility software (Meter Environment).

3.5 Tree Architecture

Single-image photogrammetry was used to capture two images of each tree: one with north-south orientation and one with east-west orientation. A scale bar with a known length was included in the photograph for reference. Image analysis was performed using ImageJ software (imagej.nih.gov/ij/) to determine tree height (H), canopy width (W), and height from ground to beginning of live canopy (HLC). H was defined as the distance from the ground to the highest branch tip, W defined as the furthest distance between two lateral branches on the same plane and HLC defined as the height of the lowest lateral branch tip. From these measurements, composite variables were created such as ratio of tree height occupied by live crown [H-HLC)/H] abbreviated as (LCR) and a width-to-height ratio capture the crown shape (CrS) seen in Figure 4.4. Final values of each tree were calculated by taking a mean of the values measured from both orientation images. The circumference of the trunk was measured 10cm above the graft union with measuring tape and values were converted to diameter (TD). As opposed to diameter at breast height, or DBH, this method controls for uneven heights of graft unions between trees which may skew the data.

3.6 Leaf Area

In July 2021, three leaf samples were collected from each ramet of cultivar. Samples were taken from random points of the canopy that could be accessed using an orchard ladder. Samples were labeled and immediately refrigerated. The LI-3000C (LI-COR Biosciences) was used to measure the leaf area of the samples to three significant digits and the number of leaflets on each leaf was recorded. Mean leaf area (LA) and mean leaflet area (LLtA) were determined from the readings.

3.7 Data analysis

One-way ANOVAs were used to check for significant differences in Tau values, phenology, architectural traits and leaf morphology between cultivars on measurement dates. Using the “agricolae” package on R, ad-hoc Fisher’s least significant different (LSD) tests were performed using an alpha of 0.05 to compare means between cultivars. A Pearson’s correlation and linear regression analysis was used to assess for predictors of the response variable Tau. This was repeated for each Tau measurement date. Variables demonstrating a relationship with Tau were incorporated into a multiple linear regression model by adding variables in descending order of their correlation with Tau. If both a composite variable and its component variable (e.g., W a component of CrS) were both correlated with Tau, only the variable with the stronger relationship was utilized. Various models were tested to draw insight on which variables explained the highest amount of variation in the response variable.

4. RESULTS AND DISCUSSION

4.1 Bud Break

The first tree initiated bud break on April 8th 2021, Julian date (jd) 98, and the final tree on May 7 (jd 127). Figure 2.5 illustrates the wide range of bud break dates observed, with significant differences between cultivars (p=<0.01). Ramets within some cultivars varied in bud break date, such as ‘Emma K’, possibly due to field-level or rootstock effects. In some cultivars, bud break timing and subsequent foliation largely avoid the critical period where winter crops are most sensitive to shade (Artru et al., 2017; Dufour et al., 2013). For example, ‘Shessler’ had developed extensive crown vegetation and nearly reached its final Tau level when ‘Myers’ had only begun to break dormancy. This high level of bud break variation is potentially due to differing physiological adaptations related to chilling hour requirements and spring frost avoidance specific to the cultivar's geographic region of origin. Most of the cultivars used in this study originated from states north of the study site and thus broke dormancy later than wild trees in the area (Michler et al., 2007).

4.2 Incidental Tau

Mean Tau values of the inner and outer-ring measurements at a single time point are defined as incidental Tau (TauI). Baseline TauI readings were not significantly different amongst cultivars prior to bud break, suggesting that woody biomass was not a driver of cultivar-specific shading effects. TauI values declined as foliage began filling the canopies (Figure 2.6) Significant differences ($p=<0.05$) in TauI were identified amongst cultivars on all measurement days but the greatest statistical support for mean separation was on May 12, julian day (jd) 132 ($p<0.01$) (Table 2.2). On this date, 'Hay', 'Myers', and 'Daniel' showed significantly higher (35.98%, 33.03% and 27.31%, respectively) TauI than 'Sparrow'. 'Shessler' and 'Tomboy' trees had developed dense canopies at this time point and had significantly less (32.29% and 27.12%) TauI than 'Sparrow'. The 'Sparrow' cultivar was chosen for comparison because it had a TauI value closest to the group mean. Although informative to understory light dynamics, TauI values can only give a snapshot of light transmittance at a specific moment in time. Furthermore, the ranking of cultivars by their TauI values tend to change somewhat at each measurement point. A more stable metric may aid in interpretation of broad patterns.

4.3 Cumulative Tau

The overall understory accumulation of PARt throughout the spring is perhaps a more useful metric in characterizing a cultivar's level of complementarity with winter crops. To quantify PARt in the aggregate, cumulative Tau (TauC) values are expressed by adding each new reading to the previous. Figure 2.7 illustrates the rise in TauC across the experimental period. Similar to TauI, there were significant differences (p=<0.05) in TauC between two or more cultivars at all measurement points, and the greatest number of significantly different groups occurred on May 12 (jd 132) in the middle of the critical period (Table 2.3). On this date, cultivars ‘Daniel’ and ‘Hay’, had significantly higher TauC (13.86% and 14.56%, respectively) than ‘Sparrow’, which again best reflected the group average. ‘Shessler’ and ‘Tomboy’ had significantly lower (22.27% and 16.83%) TauC than ‘Sparrow’. Cultivar rankings based on TauC were rather consistent at each data collection time and display a clearer pattern of each cultivar’s Tau levels throughout the experimental period. High levels of light accumulation in the understory may translate to proportional increases in growth and yield of alley crops. Data presented here can be compared with the specific photosynthetic needs of the crop species and facilitate design of plant-plant interaction studies. Additionally, the data inform selection of cultivars that provide improved understory light conditions for alley cropping winter grains and a basis for developing selection criteria in breeding. A similar approach of quantifying TauC of cultivars during the summer and autumn periods could identify other cultivar-crop combinations for effective ACS.

4.4 Tau Percentage

Tau Percentage (TauP) is a tree’s Tau measurement at a single time point relative to its baseline reading at dormancy. TauP intends to reflect the amount of shading caused only by leaves, flowers and vegetative shoots, as it adjusts for Tau at dormancy. TauP was calculated by dividing the TauI measurement of each cultivar and date by the respective Tau value at dormancy. TauP values for all ramets begin at 100% at the start of the study and decline throughout the experimental period (Figure 2.8). There were significant differences between cultivars on each measurement day except June 15 (jd 166). On May 12 (jd 132), the highest level of significant differences (p=2.08e-05) and number of significantly different groups were observed. ‘Hay’, ‘Myers and ‘Kwik Krop’ showed significantly higher (33.76%, 31.76% and 27.06%, respectively) TauP than ‘Sparrow’. Cultivars ‘Shessler’ and ‘Tomboy’ displayed significantly lower (31.62% and 23.79%) TauP than ‘Sparrow’. Cultivar rankings of TauP are similar to TauI. This observation, combined with the lack of significance at baseline readings suggests that cultivar effects on understory PARt are largely due to the phenology, density and distribution of foliage as opposed to shading effects from woody biomass. Other methods of standardizing the Tau values were attempted such as standardizing by bud break date and by trunk diameter but results showed no significance.

4.5 Cultivar Selection

When ranking cultivars by TauI, TauC or TauP there are specific cultivars that consistently show superior levels of light infiltration. While phenological and architectural traits related to complementarity may be present throughout the *J. nigra*, the data presented here suggest ‘Myers’, ‘Daniel’, ‘Sparks 127’ and ‘Hay’ have increased potential for use in ACS with spring-ripening crops such as winter wheat and barley. Nut crop productivity and quality varies amongst these cultivars. UMCA historical data shows that ‘Hay’ and ‘Sparks 127’ have high kernel percentage and overall yields (Coggeshall, unpublished). The historical data also shows that ‘Hay’ displays the most consistent productivity (i.e., low alternate-year bearing) than any other cultivar used in this study. ‘Myers’ and ‘Daniel’ have high kernel percentage but relatively low yields.

It is important to note that there were fewer significant differences in Tau on the final measurement date of June 15 (jd 166), suggesting that there may be less cultivar-specific shading effects in the summer months. While this date is past the critical period for grain filling/ripening for winter wheat in the region (jd ~115-145), it is relevant for considering summer alley crops. However, 'Sparks 127', 'Daniel', and 'Kwik Krop' had significantly less retention of foliage in the autumn, indicating that there may have been increased understory PARt during late-summer and fall. Further experimentation into PARt throughout the growing season would highlight cultivars that may be complementary with summer and fall crops. Additionally, agroforestry practices such as silvopasture (trees on pasture) and forest farming (specialty crops grown in understory) may have unique requirements for shade and sun at specific times of the growing season and would benefit from this research as well. In such cases, optimizing canopy shade for animal shelter, mushroom production or high-value specialty crops would entail corresponding tree cultivar selection.

While characterization for crown light infiltration can highlight superior cultivars, it does not explain the mechanisms driving differences seen. Identifying factors correlated with PARt levels through the tree crown may facilitate the characterization of other black walnut populations as well as inform selection criteria in breeding programs interested in complementarity traits.

4.6 Trait Analysis

The fixed-effects ANOVAs for each trait indicate that there were significant differences amongst cultivars for most architectural and phenological traits, as seen in Table 2.5. Bud break showed the greatest mean separation ($p<0.01$) followed by CrRt ($p<0.01$), H ($p<0.05$), W ($p<0.05$) and CrS ($p<.1$). In contrast, LCR and HLC, from which it is derived, were not significant, likely due to the pruning of lower branches throughout the orchard overshadowing any potential cultivar differences. Leaf morphology also showed no differences, possibly due to low numbers of samples/ramets or from non-random collection of samples in accessible areas of the canopy. Although some traits lacked differences between cultivars, there still may be tree-level associations with understory Tau values.

4.7 Pearson's Correlation

Architectural, morphological and phenological traits as well as Tau values (incidental, cumulative and percentage) were included in a Pearson's correlation analysis. Correlations were stronger when Tau was calculated using the outer ring of PAR measurements, likely due to the inner ring being saturated with shade and causing more uniform measurements across all trees. Thus, outer ring measurements were exclusively used in the correlation analysis. Strongly correlated pairs of variables are highlighted in Table 2.6. The results indicate strong negative correlations (<-0.55) between Tau and crown width, crown shape and autumn CrRt. Strong positive correlations (>0.55). were observed between Tau values and bud break. There was nearly a 0.8 correlation between TauP and bud break, as TauP is specifically associated with foliage development. Moderate but significant correlations were seen between LCR and cumulative Tau (0.479) as well as TD and TauI (0.361). Leaf and leaflet area morphology had no significant correlations with Tau.

4.8 Linear Regression

Simple linear regressions were used to test if architectural and phenological traits are predictive of incidental Tau. Traits were chosen based on having moderate or high Pearson's correlation coefficients with outer ring Tau values on jd 132 and included CrS, CrRt, LCR and bud break. The results of the linear models are plotted in (Figure 2.9). Regressions for each trait were statistically significant (p=0.0001394, p= 2.305e-05, p=0.07443, p=3.336e-08 respectively). The R^2 value was highest for bud break (0.66), suggesting it has the most important role in predicting Tau. The remainder of the traits had R^2 values below 0.5. When these variables were combined in the multiple linear regression model, there was appreciably more prediction power than any one variable alone. The fitted fixed effects regression model:

$$[TauI=0.389+CrS*-0.223+CrRt*-0.019+LCR*-0.707+BB*-0.009]$$

was significant and explained over 80% of the variation in Tau (adjusted $R^2 = 0.805$, $F(4, 26) = 31.93$, $p<0.01$). The explanatory power of this model is similar to the model developed by Mourelle (2001) that used tree species and biomass as covariates to explain 80% of variation in PARt observed in temperate hardwood species. It is possible that the residual variation is due to additional component traits not measured in this study as well as environmental factors, edge effect and rootstock influence.

The correlations and linear regression results suggest that high levels of understory light infiltration are found in trees with low values for crown width, crown shape, and live crown ratio. By choosing cultivars that exhibit these architectural traits (e.g., ‘Hay’, ‘Myers’ and ‘Sparks 127’) and by periodic pruning of outer limbs and lower branches, growers could optimize the amount of PAR reaching the understory crops. Moreover, the strong relationship between Tau and bud break supports the potential to improve complementarity with spring-ripening crops by selecting cultivars that have late bud break dates (e.g., ‘Thomas’, ‘Kwik Krop’, ‘Hay’ and ‘Myers’). The trees exhibiting early defoliation in the fall (low CrRt) also showed high spring Tau values. These shorter season length individuals present an opportunity for more complex agricultural systems using multiple alley crops that optimize the land use efficiency.

The associations observed in this experiment between architectural, phenological traits and Tau levels should be tested on additional replicated populations. Once validated, the associations could be implemented as a method to efficiently identify seedling individuals that have potential in ACS. Moreover, the trait relationships seen here in black walnut could be tested in other tree crop species such as Persian walnut (*Juglans regia L.*), Pecan (*Carya illinoinensis* Wangenh.) or Chinese Chestnut (*Castanea mollissima* Blume).

5. CONCLUSIONS

The design and successful implementation of mixed-species and agroforestry systems require the maximization of complementarity between components. In this study, we examined the characteristics of black walnut cultivars that are most relevant to aboveground competition for light. The results showed that there are black walnut nut cultivars that may be ideal for use in mixed systems where understory light during the spring is desirable. This research could be built upon by examining complementarity with different tree crop species, crop species or during different growing seasons. Moreover, correlation and linear regression analysis of component traits suggest the mechanisms for improved light infiltration including bud break date, tree shape and live crown ratio. As the dynamics of competition in ACS become better understood, researchers and growers can begin to design and implement highly productive agroforestry systems that also provide ecosystem services by the increasing landscape heterogeneity.

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Figure 2.1 Repositories A (*top)* and B (*bottom*) of black walnut cultivars were established in 1996 in New Franklin, MO.

Figure 2.2 Two adjacent canopies with contrasting phenology. ‘Shessler’ (*left*) has begun vegetative shoot expansion and catkin development throughout its canopy and ‘Thomas’ (*right*) is at an earlier stage.

Figure 2.3 Locations of the 16 PARt readings included an inner ring of 8 measurements adjacent to the trunk and an outer ring at approximately 50% of the distance to the dripline (bottom) Direct sun beams infiltrating a fully-leafed canopy at an outer-ring measurement location.

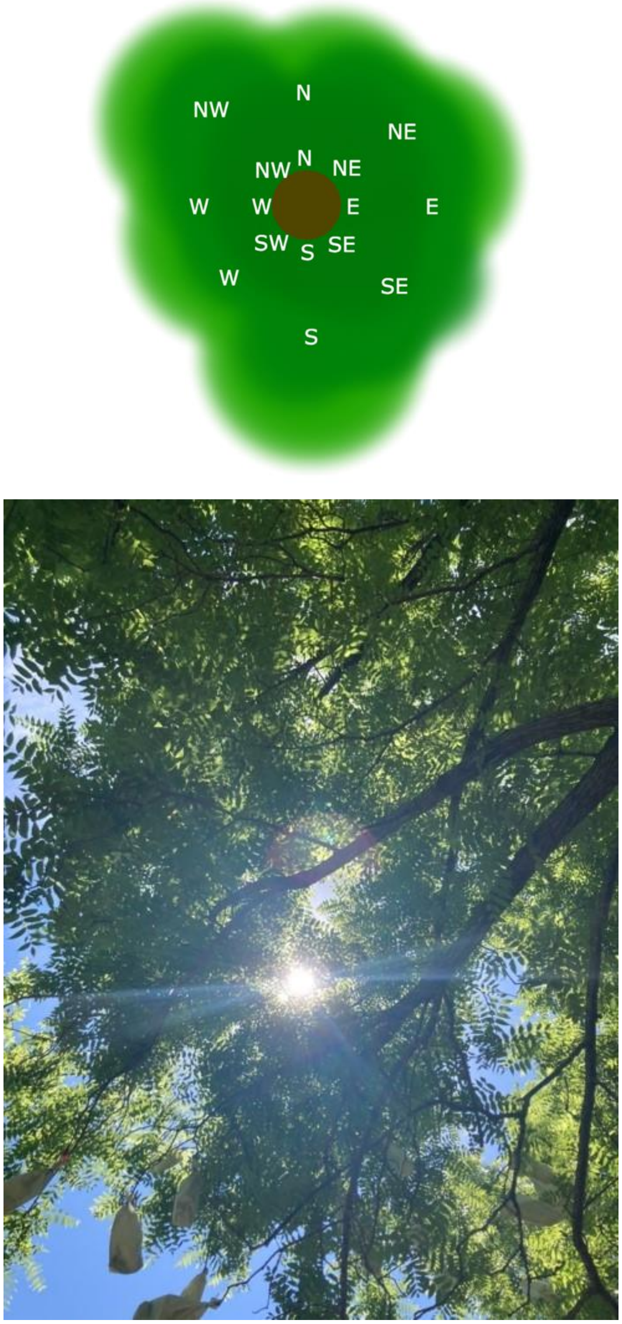

Figure 2.4 Crown architecture varies by tree due to combinations of genetic, environmental, and management. ‘Mystery’ (*top*) has higher crown shape (CrS) and live crown ratio (LCR) than ‘Thomas’ (*bottom*). Tree photos taken with reference bar for scale.

Figure 2.5 Spring phenology of each cultivar begins with bud break. Horizontal bars are white at bud break and gradually fill in green corresponding with Tau values by Julian date (jd) until June

15 (jd 166). The red box indicates critical period of wheat development and filing between April 25 (jd 115) and May 25 (jd 145).

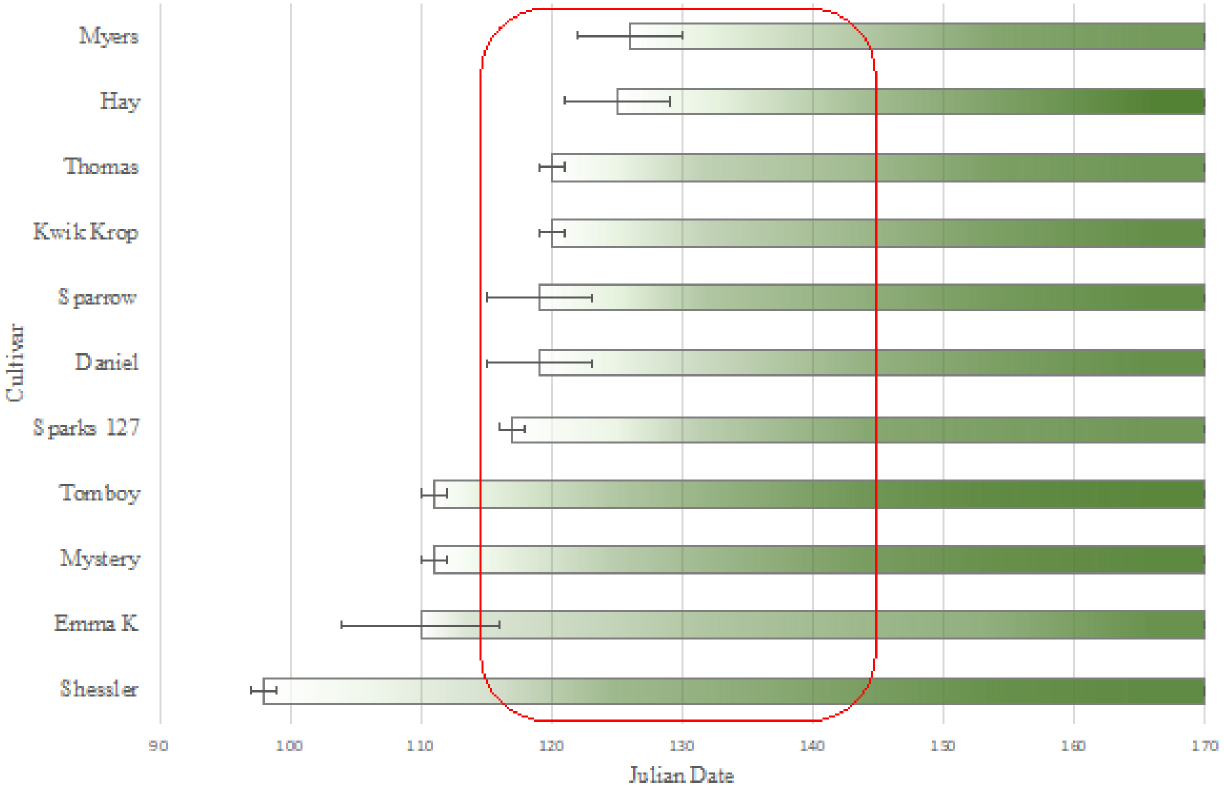

Figure 2.6. Incidental Tau values by julian date (jd)of each cultivar from April 15 (jd 115) to June 15 (jd 166). The 95% confidence intervals and LSD groups are provided in the legend based upon jd 132.

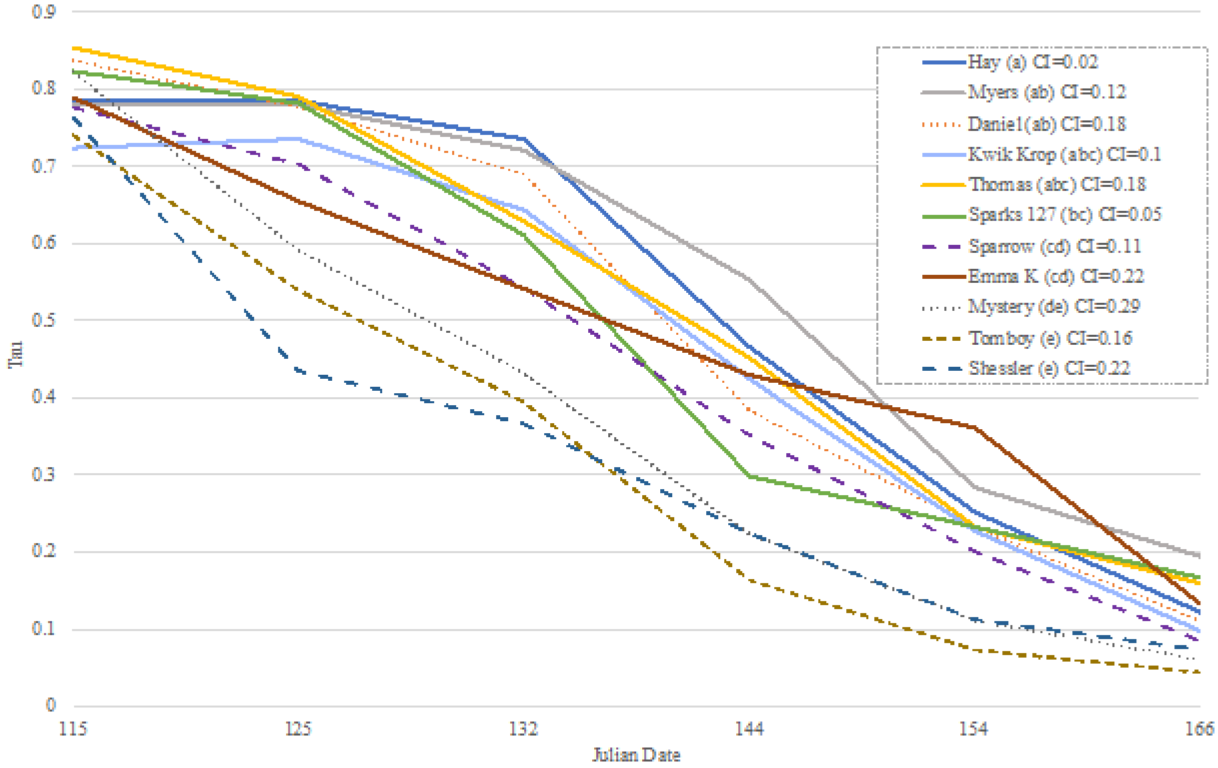

Figure 2.7. Cumulative Tau values by Julian date (jd) of each cultivar from jd 115-165. Values represent the accumulation of PAR throughout the experimental period. The 95% confidence intervals and LSD groups are provided in the legend based on jd 132.

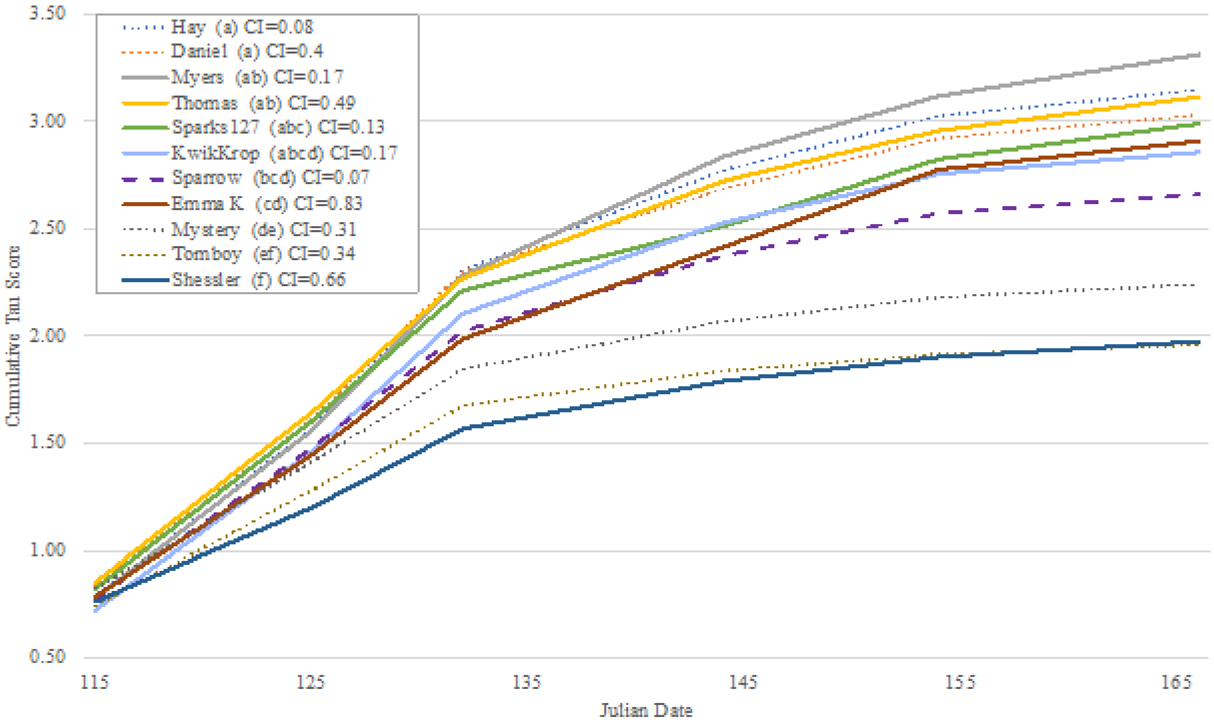

Figure 2.8 Tau percentage (TauP) values by Julian date (jd) of each cultivar between jd 115-165. All ramets were assigned 100% at dormancy and values decline with foliation of the crown over time. The 95% confidence intervals and LSD groups are provided in the legend based on jd 132.

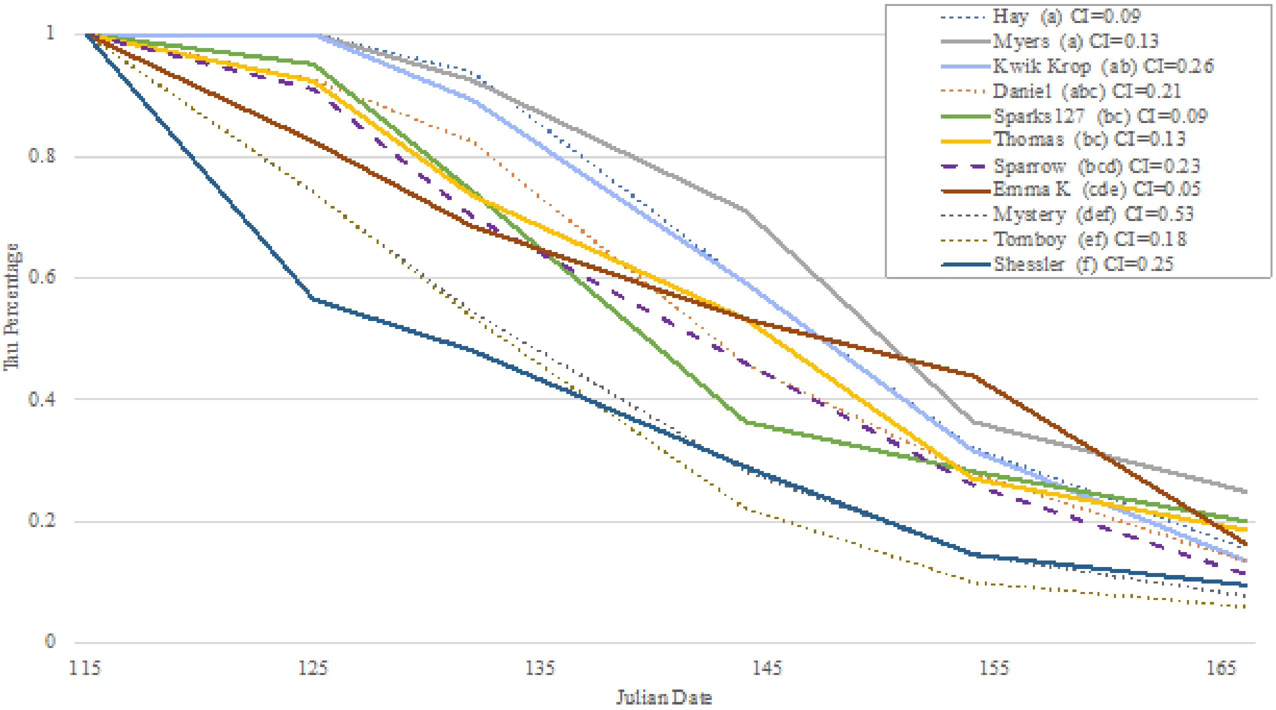

Figure 2.9 Linear models regressing TauI on May 12 (jd 132) with crown shape (a), crown retention (b), live crown ratio (c) and bud break date (d).

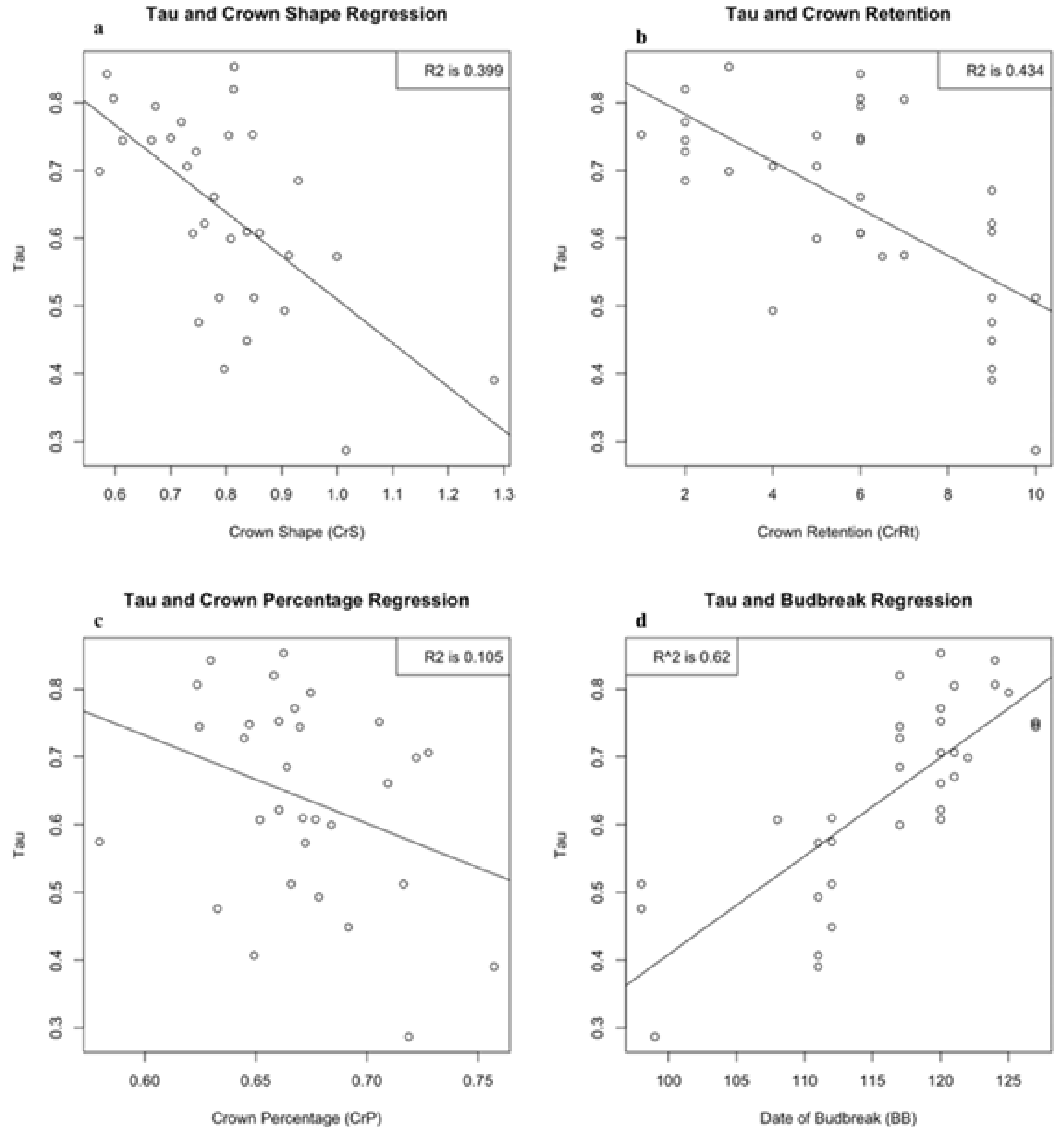

Table 2.1 The geographic origin, bud break season and synonyms of the studied cultivars. In addition, historical data on nut quality and total yields collected 2002-2009. Kernel percentage refers to the amount of edible kernel relative to overall nut weight and alternate bearing index (ABI) reflects the extent of alternate-year bearing observed.

Cultivar	Origin	Budbreak	Synonym	Nut Mass	Kernel %	NutYield	A.B.I.
Daniel	Ohio	Mid	Sauber	21.98g	30.42%	1827	0.61
Emma K	Illinois	Early		17.46g	32.74%	5285	0.76
Hay	Missouri	Late		22.93g	31.33%	3291	0.22
KwikKrop	Kansas	Mid	Boellner	17.64g	31.79%	1320	0.40
Myers	Ohio	Late	Elmer Meyers	14.89g	33.10%	499	0.55
Mystery		Mid		18.13g	27.26%	2415	0.47
Shessler	Ohio	Early		20.48g	27.91%	3169	0.59
Sparks127	Iowa	Mid		15.08g	33.94%	2765	0.56
Sparrow	Illinois	Mid		17.08g	26.77%	4760	0.32
Thomas	Pennsylvania	Mid		23.29g	23.90%	1247	0.56
Tomboy	Missouri	Early		21.08g	23.28%	2094	0.80

Table 2.2 Incidental Tau values by cultivar from jd 124-166 with p-values from one-way ANOVA and Fisher's LSD groups. Cultivars with the same letter are not significantly different.

Cultivar	jd 124	jd 132	jd 145	jd 154	jd 166
Hay	0.785 (a)	0.737 (a)	0.466 (ab)	0.252 (a)	0.122 (abc)
Myers	0.780 (a)	0.721 (ab)	0.553 (a)	0.284 (a)	0.195 (a)
Daniel	0.776 (a)	0.690 (ab)	0.384 (abc)	0.233 (ab)	0.111 (abc)
Kwik Krop	0.736 (a)	0.643 (abc)	0.425 (ab)	0.228 (ab)	0.098 (abc)
Thomas	0.790 (a)	0.628 (abc)	0.452 (ab)	0.233 (ab)	0.160 (ab)
Sparks 127	0.782 (a)	0.610 (bc)	0.299 (bcd)	0.309 (a)	0.167 (ab)
Sparrow	0.703 (ab)	0.542 (cd)	0.352 (bc)	0.201 (ab)	0.085 (bc)
Emma K	0.655 (abc)	0.541 (cd)	0.43 (ab)	0.292 (a)	0.133 (abc)
Mystery	0.592 (bc)	0.432 (de)	0.223 (cd)	0.112 (bc)	0.061 (c)
Tomboy	0.540 (cd)	0.395 (e)	0.164 (d)	0.073 (c)	0.044 (c)
Shessler	0.435 (d)	0.367 (e)	0.225 (cd)	0.113 (bc)	0.073 (bc)
p Value	p=<0.01	p=<0.01	p=<0.01	p=<0.01	p=<0.01

Table 2.3 Cumulative tau values by cultivar from Julian date (jd) 124-166 with Fisher's LSD groups. Cultivars with the same letter are not significantly different.

Cultivar	jd 124	jd 132	jd 145	jd 154	jd 166
Hay	1.57 (abc)	2.31 (a)	2.77 (ab)	3.03 (ab)	3.15 (ab)
Daniel	1.61 (ab)	2.30 (a)	2.69 (abc)	2.92 (ab)	3.03 (ab)
Myers	1.56 (abc)	2.28 (ab)	2.83 (a)	3.12 (a)	3.31 (a)
Thomas	1.64 (a)	2.27 (ab)	2.72 (abc)	2.96 (ab)	3.12 (ab)
Sparks 127	1.60 (ab)	2.21 (abc)	2.51 (abc)	2.82 (ab)	2.99 (ab)
Kwik Krop	1.46 (abcd)	2.1 (abcd)	2.53 (abc)	2.76 (ab)	2.85 (ab)
Sparrow	1.48 (abc)	2.02 (bcd)	2.37 (cd)	2.58 (bc)	2.66 (bc)
Emma K	1.44 (bcd)	1.99 (cd)	2.42 (bcd)	2.78 (ab)	2.91 (ab)
Mystery	1.41 (cd)	1.85 (de)	2.07 (de)	2.18 (cd)	2.24 (c)
Tomboy	1.28 (de)	1.68 (ef)	1.84 (e)	1.91 (d)	1.96 (c)
Shessler	1.20 (e)	1.57 (f)	1.79 (e)	1.9 (d)	1.98 (c)
p Value	p<0.01	p<0.01	p<0.01	p<0.01	p<0.01

Table 2.4 Tau percentages by cultivar from Julian date (jd) 124-166 with Fisher's LSD groups. Cultivars with the same letter are not significantly different.

Cultivar	jd 124	jd 134	jd 145	jd 154	jd 166
Hay	1.00 (a)	0.939 (a)	0.591 (ab)	0.320 (ab)	0.154 (abc)
Myers	1.00 (a)	0.925 (a)	0.710 (a)	0.364 (ab)	0.249 (a)
Kwik Krop	1.00 (a)	0.892 (ab)	0.591 (abc)	0.316 (abc)	0.134 (bc)
Daniel	0.926 (ab)	0.824 (abc)	0.458 (abc)	0.279 (abc)	0.134 (abc)
Sparks 127	0.952 (ab)	0.742 (bc)	0.363 (bcd)	0.374 (a)	0.200 (ab)
Thomas	0.923 (ab)	0.735 (bc)	0.532 (ab)	0.27 (bcd)	0.185 (ab)
Sparrow	0.910 (ab)	0.702 (bcd)	0.460 (abc)	0.26 (abcd)	0.112 (abc)
Emma K	0.825 (b)	0.684 (cde)	0.532 (abc)	0.438 (ab)	0.164 (abc)
Mystery	0.739 (b)	0.544 (def)	0.282 (cd)	0.144 (cd)	0.077 (bc)
Tomboy	0.741 (b)	0.535 (ef)	0.221 (d)	0.099 (d)	0.060 (c)
Shessler	0.565 (c)	0.480 (f)	0.290 (cd)	0.145 (cd)	0.095 (bc)
p Value	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p>0.1$

Table 2.5 Architectural (top), phenological and leaf morphological (bottom) data collected from 11 black walnut cultivars. Values are given with 95% confidence intervals. Below cultivar values is the p value for the trait along with a descending ranking of cultivars with LSD letters. Cultivars sharing a letter are not significant.

Cultivar	Width (m)	Ht (m)	CrS	LCR	TD (cm)
Daniel	7.88 (bcd)	10.03 (ab)	0.79 (abc)	0.68 (abc)	43.74 (ab)
Emma K	8.03 (bcd)	9.68 (ab)	0.83 (abc)	0.64 (c)	47.45 (a)
Hay	6.61 (cd)	9.51 (abc)	0.69 (bc)	0.67 (bc)	36.84 (bc)
Kwik Krop	8.07 (abcd)	9.94 (ab)	0.81 (abc)	0.67 (bc)	43.52 (ab)
Myers	6.49 (d)	10.22 (ab)	0.63 (c)	0.65 (c)	36.84 (bc)
Mystery	10.19 (a)	10.55 (a)	0.97 (a)	0.71 (ab)	45.22 (ab)
Shessler	8.64 (abc)	9.97 (ab)	0.87 (ab)	0.67 (bc)	40.55 (abc)
Sparks 127	6.63 (cd)	8.49 (c)	0.78 (abc)	0.64 (c)	32.59 (abc)
Sparrow	7.49 (bcd)	9.58 (ab)	0.78 (abc)	0.68 (abc)	44.69 (c)
Thomas	8.35 (ab)	10.31 (a)	0.80 (a)	0.70 (a)	-
Tomboy	8.31 (abcd)	9.22 (bc)	0.90 (a)	0.67 (bc)	42.89 (ab)
p Value	$p<0.0542$	$p<0.05$	$p<0.10$	$p<0.10$	$p<0.1$

Table 2.5 cont'd

Cultivar	LA(cm2)	LtA(cm2)	Bud break	CrRt
Daniel	294.42	21.99	119.00 (bc)	0.30 (d)
Emma K	304.91	18.76	110.00 (d)	0.73(abc)
Hay	304.96	18.00	125.00 (a)	0.57 (c)
Kwik Krop	278.06	15.48	120.00 (b)	0.30 (d)
Myers	288.70	18.49	126.00 (a)	0.60 (c)
Mystery	332.23	18.02	111.67 (d)	0.90 (ab)
Shessler	218.38	14.86	98.00 (e)	0.97 (a)
Sparks 127	241.01	17.03	117.00 (c)	0.20 (d)
Sparrow	251.97	15.18	119.00 (bc)	0.67 (bc)
Thomas	-	-	120.00 (b)	0.70 (bc)
Tomboy	279.10	17.83	111.00 (d)	0.63 (c)
p Value	p>0.1	p=>0.1	p<0.01	p<0.01

Table 2.6 Pearson's correlations between outer ring TauI, TauC and TauP on May 12 (jd 132) and nine phenological and morphological traits of black walnut. Values approaching 1 are blue in color, values near zero are white and values approaching -1 are red in color. ***: p<0.001, **:p<0.01, *: p<0.05 . H-Height, W-Width, HLC-Height to Live Crown, CrS-CrownShape, LCR-Live Crown Ratio, LtA-Leaflet Area, LA-Leaf Area, BB-Bud Break Date, CrRt-Crown Retention, TD-Trunk Diameter, TauI-Incidental Tau, TauP-Tau Percentage, TauC-Cumulative Tau

	W				
H	0.415*	H			
HLC	-0.071	0.509**	HLC		
CrS	0.924***	0.041	-0.290	CrS	
LCR	0.376*	0.118	-0.794***	0.366*	LCR
LtA	0.012	-0.049	-0.258	0.051	0.268
LA	0.163	0.036	-0.339	0.175	0.423*
BB	-0.457**	-0.011	0.007	-0.505**	-0.025
CrRt	0.402*	0.355	0.093	0.292	0.153
TD	0.520**	0.362*	-0.013	0.430*	0.279
TauI	-0.556**	0.032	0.289	-0.631***	-0.324
TauP	-0.580***	0.073	0.242	-0.670***	-0.231
TauC	-0.585***	-0.039	0.379*	-0.640***	-0.479**

	LtA					
LA	0.852***					
BB	0.216	BB				
CrRt	-0.297	-0.528**	CrRt			
TD	0.258	-0.253	0.198	TD		
TauI	0.090	0.786***	-0.710***	-0.361*	TauI	
TauP	0.100	0.798***	-0.600***	-0.359*	0.870***	TauP
TauC	0.010	0.646***	-0.554**	-0.357*	0.875***	0.741***

CHAPTER 3: PERFORMANCE TRIAL OF SEVEN FIRST-GENERATION BLACK WALNUT BREEDING SELECTIONS FOR KERNEL PRODUCTION

1.1 ABSTRACT

The development of underutilized temperate tree crops will aid increasing regional food production capacity throughout the temperate U.S. Eastern Black walnut (*Juglans nigra* L.) is a deciduous hardwood native to the eastern North America and is well-positioned for genetic enhancement as a nut crop. Extensive germplasm resources have been assembled for this species but there have yet to be commercial releases of superior cultivars. In this trial, we evaluate the performance of the first generation of black walnut selections for improved nut production. The findings presented here demonstrate how genetic gain can be achieved within this species in key commercial traits: kernel percentage, kernel mass, in-shell yield and kernel yield. In some cases, we found selections were able to significantly outperform the parental generation in these traits by as much as 135%. Furthermore, correlation analysis incorporating tree physiology and nut morphology offer insight into the variation driving these improvements and may inform future breeding. Additional years of harvest data as well as replication sites for this trial will help refine the understanding of the commercial performance of selections and allow for broader recommendations to growers at large.

2.1 INTRODUCTION

Eastern black walnut (*Juglans nigra* L.) is an economically important hardwood species endemic to North America. Regional markets for the species' nuts and kernels provide an unique economic opportunity for smallholder farms, but to date, the crop supply is predominantly based on nuts harvested from wild trees. The regional supply reaches approximately 9.5 M kilograms, with 5-7 M kilograms typically originating in Missouri (Hammons Products, personal comm.). The volume of crop from improved orchards is low, ranging from about 9,000 to 18,000 kilograms from year to year. The total value of the kernel supply is estimated between \$2.6 and

$3.5 million annually, although revenue is also generated from the many millions of pounds of shell via various fine grit products for industrial processes. The reliance on supply from wild trees limits regional industry growth. The supply ceiling is capped and varies greatly due to masting. Further, nuts from wild trees can exhibits low kernel yield and quality. Increasing the ability to cultivate orchards with genetically improved cultivars would benefit both the industry and growers alike.

In the United States, Persian walnut (*Juglans regia* L.) is grown on 440,000 acres producing an annual crop of nuts valued at nearly $1.3 billion (USDA, 2020). This industry is limited predominantly to California due to the mild climate. There is potential to expand walnut kernel production in the Midwest U.S. by exploiting eastern black walnut (Reid et al., 2009), especially with the increasing pressures of climate change on the California horticulture industry. Like Persian walnut, black walnut produces nutritious edible nuts high in protein and omega-3 fatty acids (Câmara & Schlegel, 2016) and are used regionally in baked goods and ice cream (Wendholt Silva, 2016). Current research suggests that black walnut consumption may have protective effects against cardiovascular disease, neurodegeneration and various cancers (Câmara & Schlegel, 2016; Vu et al., 2020). Commercially, it is grown in timber plantations both in the U.S. and abroad (McKenna & Coggeshall, 2018; Šálek & Hejcmanová, 2011), but there are challenges that have limited its adoption as a nut crop in the United States.

Most wild black walnut trees produce nuts with an edible kernel percentage of 15% or less (Coggeshall, 2011). By comparison, kernel percentages in Persian walnuts can exceed 50% in California orchards (Ebrahimi et. al, 2011). Moreover, kernels from wild black walnuts generally have characteristics that undesirable to consumers such as a dark color, strong flavor and a tendency to break easily during extraction (Sideli et al., 2020; Warmund, 2008). The species is also susceptible to anthracnose, a fungal infection (Figure 3.1) caused by Gnomonia leptostyla leading to leaf defoliation during the final stage of nut ripening and alternate-year bearing (Schneider et al., 2019). Although there are black walnut kernel cultivars available today, they are predominantly wild selections or open-pollinated seedlings thereof (Zhao et al., 2018). Market prices for in-shell nuts of certain cultivars with kernel percentage >30% can reach $1.65/kg (Hammons Products, personal communication), but the trees may yield inconsistently or produce a low number of nuts. To meet the preferences of both black walnut growers and consumers, the development of improved cultivars is necessary.

Over the last 25 years, numerous genetic resources were established at the University of Missouri Center for Agroforestry (UMCA) to improve eastern black walnut for kernel production. These resources include a diverse collection of 65 named cultivars (typically of on-farm origin), >1200 breeding offspring, the first mapping population for the species, and a breeding selection trial. Approximately 1500 progeny were produced from 2002 to 2008, through controlled pollinations and paternity exclusion analysis with Short Sequence Repeat markers, to comprise the program's first breeding generation. Seedlings derived in 2002 were thoroughly characterized from 2007-2010, starting when seedlings reached five years old. Evaluations included attributes related to kernel quality (e.g., mass, percentage, color, fill, venation) and yield parameters such as total in-shell mass, quantity of nuts produced, yield efficiency, and alternate-year bearing habit. Five traits were selected to include into a ranked based index, from which the 12 highest indexing off-spring were selected to carry forward in replicated evaluations (Table 3.1)

Here, performance trial data is reported to compare yield and nut quality characters of seven of these UMCA selections with commercial cultivars as standards. The data reported here are an early basis for comparison and include that from years 2017 and 2021. Additional years of evaluation are needed to assess trait stability over time and add power to the interpretation of performance compared to standards. However, these initial data offer valuable early insight to performance, as growers are eager to adopt first generation test orchards of east black walnut for kernel production. Additionally, grafted trees of the selections' parents were included in the trial to allow opportunity for a partial diallel analysis of trait inheritance and parental combining ability, which provides valuable insight for pairing breeding parents and future crossing scheme design.

3. METHODS AND MATERIALS

3.1 Study Site

The study plot is 2.3 acres (0.93 hectare) of menfro silt loam soil located at the Horticultural and Agroforestry Research Center in New Franklin, Missouri, (39°00 N, 92°46 W) with a slope ranging from 5% to 35% (Figure 3.2). The site was mowed regularly and a broad spectrum non-selective herbicide was applied in 1-meter strips along the tree lines at least once a year. One-hundred pounds of actual N, in the form of urea, was applied annually with 60 pounds applied in the spring and 40 in the fall, although the fields were not fertilized every year.

3.2 Plant Materials

A population of 157 grafted trees was established in a randomized block design in 2010. The population included 3-5 ramets of parental cultivars and UMCA selections. Additional progeny of the parental cultivars were also included in the population for the partial diallel analysis for a total of 31 full-sibling families. Since the establishment of the site, there has been mortality of the trees due to damage from mechanized equipment, voles and disease. The 2007-2010 selections were included in the performance trial and summarized by pedigree and number of ramets in Table 3.2.

3.3 Phenology Data Collection

Every week beginning in mid-April 2021, trees were monitored for signs of budbreak at distal end of the branches. The trees were classified according to the 5-stage scale, a method commonly used in hardwood tree phenology research (Gričar et al., 2017; Pang et al., 2021) where 1 is dormant and 5 is full leaf unfolding (Figure 3.3). Bud break was defined as date where 50% of the canopy was in stage 4—leaf expansion. Determination of fruit ripening was more subjective and involved squeezing the fruits by hand and recording the ripe date (RD) when 50% of the fruits could be easily dented from pressure. If fruits dropped from the tree before becoming soft, date of 50% fruit drop was used as RD instead. Values for season length (SL) were created by subtracting bud break from RD and represent the amount of days between the tree's bud break date and when its nuts were harvested. This method differs slightly from the "IPGRI: Descriptors of *Juglans spp.*" (IPGR, 1994) method which measures season length starting from first pistillate flower receptivity. Anthracnose susceptibility (AnthS) was evaluated in late August by visually classifying tree canopy infection levels on a scale of 1-5 with high values indicating necrosis (Figure 3.1) throughout the canopy. AnthS and bud break were collected in 2020 and 2021 and a mean value by ramet was used for analysis.

3.4 Yield Performance Data Collection

Yield performance was recorded only in 2021. Nuts were manually harvested from the trees on their RD by shaking the trunk and branches. If some nuts would not dehisce from the branches, they were collected on a later date. The final nut quantity was recorded for each tree. The nuts were promptly dehulled using a commercial dehuller and pressure-washed before wet in-shell yield mass was recorded to the nearest 10g using a digital scale. The nuts were then air-dried for approximately 3 weeks and then refrigerated until they were ready to be evaluated.

3.5 Nut Quality Data Collection

Subsamples of 10 nuts per ramet were evaluated for nut quality attributes. Each nut was weighed after removing any dried hull material still attached to the shell. Nuts were then cracked using a “Master Cracker” in 2017 and a “Grandpa’s Goody Getter” in 2021. The quantity of kernel quarters retrieved from each nut after the first crack was recorded on a scale of 0-4. The kernel was then removed from the shell and weighed. Kernel color was graded on a scale of 1-5: 1—light pale-yellow, 2—tan, 3—light brown, 4—dark brown, 5—black. Visual appearance of veins in the kernel was graded on a scale of 1-4 with low values indicating lack of noticeable venation and high values indicating dark and thick veins. In addition, the plumpness of the kernel (kernel fill) was graded on a scale of 1-5 in 2017 and a scale of 3-7 in 2021, following the methods outlined in “IPGRI: Descriptors of *Juglans spp.*” (IPGR, 1994). The dimensions of the nuts were measured with digital calipers to the nearest 0.01cm. Nut length (NEE) was measured from distal end to proximal end of the nut, nut width was measured from suture to suture (WSS) and also from cheek-to-cheek (WCC). For the purposes of this study, individual nuts exhibiting poor quality (less than 5 kernel fill value or less than 25% kernel percentage due to shriveling) were discarded, so that genetic potential of the individual for kernel mass and kernel percentage traits was accurately represented. A mean value for each nut quality trait was calculated by ramet for analysis. A composite variable of "kernel yield" was created by multiplying in-shell yield by kernel percentage represent the ability of an individual to produce high amounts of kernel.

3.6 Data Analysis

To assess genetic gain in the progeny selections, parental cultivars were chosen as benchmarks to compare the performance of the selections. Criteria of choosing these cultivar standards were that they parented at least one of the selections in the population, had at least 3 surviving ramets represented within the population, and showed exceptional historical performance in yield parameters, nut quality or both. They were included with the selections in one-way analysis of variance (ANOVA) and subsequent Fisher's LSD tests ('agricolae' package in R) to investigate significant differences with progeny selections.

The cultivar 'Sparrow' was chosen as the cultivar standard for total productivity. It was discovered in 1935 in Lomax, IL (40.34°*)* as a wild seedling. Historical data (2002-2009) collected in the UMCA cultivar repository supports the genotype is highly productive, performing in the 95th percentile of cumulative yields in both number of nuts and in-shell mass during that period. It also has a low alternate-bearing behavior indicated by its consistent year-to-year production. The cultivar 'Neel' was selected by Robert Neel in Barboursville, WV (38.41°). It is a late-leafing cultivar and produces nuts with the highest kernel mass (7.68g ± 0.75 CI 95%) observed in the cultivar collection. It was used as the cultivar standard for kernel mass. The cultivar 'Sparks 147' was selected in Beaver, IA (42.04°) by Archie Sparks for its thin shell and well-filled nuts. It has a distinct shape with peaks on both the distal and proximal ends of the nut. 'Sparks 147' consistently shows the highest kernel percentage in the collection (36.17% ± 7.48

CI 95%) and was used as the standard for this character. However, ‘Sparks 147’ has poor yields historically at UMCA.

3.7 Partial Diallel Analysis

The presence of replicated parental cultivars and progeny in this population allows for a partial diallel analysis to estimate the general combining ability (GCA), specific combining ability (SCA) as well as broad and narrow-sense trait heritability (H^2, h^2) in the clonal black walnut population. The GCA of a parent represents its breeding value for a particular trait and is associated with additive genetic effects, and SCA represents the relative performance of a cross based on dominant or epistatic effects (Rukundo et al., 2017). Broad sense and narrow sense heritability measure the level of genetic variance in a trait relative to total phenotypic variance of the population and can be used to estimate phenotype of the progeny. Analysis of variation was employed with Griffing’s method via the r package “DiallelAnalysisR”. Nut quality data collected in 2017 from 7 parental cultivars and 23 progeny was used for the analysis.

4. RESULTS AND DISCUSSION

4.1 Yield Performance

There were significant differences ($p<0.01$) between the selections and the cultivar standard ‘Sparrow’ in total in-shell yield mass with selection production ranging from 2.60kg-20.22kg. In 2021, selections ‘02.1053’, ‘02.1075’ and ‘02.1055’ respectively yielded 19.79kg, 15.97kg, 13.43kg (Table 3.3), representing yielded increases of 103%, 79% and 61% compared to ‘Sparrow’, respectively. Significant differences ($p<0.05$) were also observed for the total number of nuts produced (Table 3.3), again with selections ‘02.1053’, ‘02.1075’ and ‘02.1055’ producing the greatest number of nuts, 1,089, 727 and 602, respectively. These yields represent 138.60%, 59.08% 31.80% more nuts than ‘Sparrow’. The three UMCA black walnut selections highlighted here show exceptional performance compared to the parental generation. All other selections did not demonstrate significant difference between ‘Sparrow’ in in-shell yield nor nut quantity. While high production is important, the quality of nuts is the main determinant of the wholesale price they can command from buyers.

4.2 Kernel Percentage

In 2017, mean kernel percentages of the selections ranged from 31.095% - 38.32%, and in 2021, the range was 28.01%-35.43%. There were significant differences ($p<0.01$) in both years between the UMCA selections and cultivar standard ‘Sparks 147’ which had kernel percentages 38% and 35% in 2017 and 2021, respectively (Table 3.3). Selections ‘02.1053’ and ‘02.1002’ were significantly similar to the cultivar standard in both years while selections ‘02.1114’ and’02.1055’ were only similar in one year. All other selections were significantly lower in kernel percentage than the cultivar standard both years. Although no selections showed significant improvements in kernel percentage relative to the cultivar standard, it should be highlighted that ‘Sparks 147’ has the highest kernel percentage in the parental generation, and thus is a highly strict cultivar standard. Selections with genetic backgrounds unrelated to ‘Sparks 147’ and equivalent kernel quality, such as ‘02.1053’ and ‘02.1114’, represent tangible improvements in this trait compared to their respective parents. Furthermore, these selections were shown in the previous section to have exceptional yield performance, while ‘Sparks 147’ historically yields below the 40th percentile of the UMCA collection – positioning the mentioned selections as overall improvements across both traits.

4.3 Kernel Mass

In 2017, mean kernel mass of the selections ranged from 3.90g-6.12g (Table 3.3). Selection ‘02.1114’ produced the highest kernel mass of any selection or cultivar at 6.12g. Compared to the cultivar standard ‘Neel’, ’02.1114’ produced 0.88g (16.80%) more kernel mass ($p<0.01$). Most selections produced a kernel mass not significantly different than the cultivar standard. However, kernel mass of ‘02.1005'' and ‘02.1022’ was significantly lower than the cultivar standard ‘Neel’ ($p<0.01$). In 2021, mean kernel mass of the selections were slightly lower and ranged from 3.23g-5.50g. Selections ‘02.1002’, ‘02.1075’ and ‘02.1053’ produced nuts with kernel mass not significantly different than ‘Neel’. The remaining selections had kernel mass values significantly lower than ‘Neel’. Selections ‘02.1002’ and ‘02.1053’ are noteworthy as they produce nuts with both kernel percentage and mass that meet or exceed the respective cultivar standard. Multivariate analysis of historical data in the UMCA cultivar repository indicates that high kernel percentage does not imply high kernel mass (Meier, unpublished). In fact, the trait eigenvectors are orthogonal in principal components analysis, meaning they are supported by distinct variation. Consequently, selections ‘02.1002’ and ‘02.1053’ could be considered unique in that they express both characters to a high degree.

4.4 Kernel Yield

Greater mean separation was observed between individuals in the composite variable of kernel yield compared to either kernel percentage or in-shell yield (Table 3.3). In 2021, the selections ‘02.1053’ and ‘02.1075’ yielded 6.66kg and 5.20kg of kernel fresh weight. Compared to the cultivar standard for kernel yield ‘Neel’ (2.84kg), this equates to a 134.51% and 83.01% respective improvement ($p<0.01$). Selections ‘02.1114’ and ‘02.1055’ performed as well as

‘Neel’ in kernel yield, and ‘02.1002’, ‘02.1022’ produced similarly to the cultivar standards. Kernel yield may be of high interest to commercial growers as it quantifies the actual production of sellable nut meat. In addition, it helps to highlight the advancements made in this generation of selections compared to the parental generation. However, since eastern black walnut is a masting species with variable inter-annual yields, the exceptional kernel yield observed in ‘02.1053’ and ‘02.1075’ should be averaged across at least 3 years of data to provide more precise predictions of future performance.

4.5 Additional Nut Quality

Selections in this trial showed pellicle color that were either similar or slightly darker than cultivars, however rankings were highly variable between 2017 and 2021 (Table 3.3). While uniform harvesting and post-processing practices were performed, it is possible that delays in hulling could have influenced pigmentation of the nuts. Such delays are the single most important factor in determining pellicle color. In 2021, kernel fill ‘02.1053’, ‘02.1055’ and ‘02.1075’ showed highest values (6.67, 6.30 and 6.11, respectively). Considering that these selections also yielded the highest number of nuts in the trial, it appears that the high yields of these trees does not come at the detriment of nut fill. Comparison of fill between the years is difficult due to different phenotyping methods and little mean separation was seen in 2017. Differences were observed in quarters in 2017 with ‘02.1055’ and ‘Football’ showing highest scores (3.15 and 3.32 respectively). Selection ‘02.1053’ had relatively low quarters, a possible trade-off for its high yields. For kernel veins, ‘02.1005’ and ‘02.1075’ showed the lowest amount of detectable veins while ‘02.1022’, ‘02.1053’ and ‘02.1055’ were amongst the highest group. There were no observed differences in quarters nor kernel veins in 2021.

4.6 Trait Correlations

A Pearson's correlation analysis was performed on traits related to nut attributes, yield, phenology and anthracnose susceptibility. The analysis, seen in Table 3.4, was performed on 2020 data of 30 individuals (parents, elite and non-elite progeny) where data points were available for every trait. Spring bud break date was only significantly correlated with RD, and the relationship was weak (0.276). Moderate but significant positive correlations were found between SL and kernel mass/percentage/fill, possibly reflecting the long season necessary to produce large nuts of high quality. SL, however, was not associated with any yield parameters. Moderate but significant positive relationships were found between in-shell yield and nut quality traits such as kernel mass, kernel fill and WCC. There was a weak but significant negative correlation between kernel percentage and quartering rate. Kernel mass and nut dimensions (WCC, WSS, LEE) were negatively correlated with anthracnose susceptibility.

Nut dimensions (WCC, WSS, LEE) were also moderately correlated (0.51-0.623) with nut quality parameters such as kernel percentage, kernel mass and quarters. The dimension with the highest significant correlation (0.571) with kernel percentage was nut LEE. The correlation with kernel percentage was strengthened to 0.623 when NEE was expressed in relation to WSS (LEE / WSS). These relationships highlight the contribution of nutshell morphology to the high variability of kernel percentage seen in black walnut cultivars and provide additional selection criteria for breeders. If the associations of nut dimensions with kernel percentage seen here are validated, they may allow for swifter phenotyping, potentially reducing the need for time-consuming cracking and weighing of nut meats It should be noted, however, that the sample population was relatively small (n=30) and included only individuals previously identified to have high kernel quality. Kernel pellicle color and venation had no significant correlations with any other trait. Although nuts were harvested and hulled as promptly as possible, color has been shown to be affected by delayed hulling of the nuts after ripening (Warmund, 2008).

4.7 Partial Diallel Analysis

Due to small family size and tree mortality reducing ramet number, statistical power is limited. Nevertheless, the results show significant differences ($p < 0.1$) between parental cultivars in GCA (Table 3.5) and between crosses in SCA (Table 3.6). ‘Sparks 147’ and ‘Brown Nugget’ were significantly lower in their GCA of nut mass than other cultivars. The SCAs of nut mass showed no significance between crosses. The GCAs of kernel mass in ‘Daniel’ and ‘Neel’ were significantly higher than those of ‘Rupert’ and ‘Brown Nugget’. The SCA of the ‘Sparrow’ x ‘Emma K’ cross was significantly higher than other crosses. Cultivars ‘Daniel’, ‘Emma K’ and ‘Neel’ were high in kernel mass GCAs and were part of high SCA crosses. The GCAs of kernel percentage in ‘Sparks 147’, ‘Daniel’ and ‘Emma K’ were significantly higher than other cultivars. These cultivars were also members of crosses that were high in SCA for kernel percentage

For kernel pellicle color, a trait where low values are desirable, ‘Spark 127’ showed higher GCA and ‘Football’ showed lower values compared to other cultivars. No crosses showed significant differences in SCA for pellicle color. ‘Brown Nugget’ x ‘Brown Nugget’ showed higher SCA for quarters than ‘Sparrow’ x ‘Brown Nugget’ and there was no significant GCA for this trait. For kernel venation, a trait where low values are desirable, ‘Sparrow’ x ‘Emma K’ and ‘Sparrow’ x ‘Rupert’ showed lower venation than other crosses. There were no GCA or SCA differences in kernel fill. It is important to highlight that this analysis identified the ‘Sparrow’ x ‘Emma K’ cross to have significantly higher SCA in kernel mass, kernel percentage, nut mass and lower venation than other crosses. This was found despite these cultivars not showing highest values in GCA for these traits. A possible explanation for this observation would be desirable combinations of dominance effects in these traits when these cultivars are crossed.

4.8 Trait Heritability

There was relatively high (0.69) H^2 found (Table 3.7) for kernel percentage as well as kernel mass (0.504) and quarters (0.52) indicating that genetic background explains the majority of the variation found in these traits. This suggests that environmental effects are less pronounced, particularly in kernel percentage and that selection for this trait will result in accelerated genetic gain. All other traits had H^2 lower than 0.5 and h^2 values for all traits were lower than 0.5 as well suggesting more environmental effects controlling the variation.

5.1 CONCLUSIONS

After just one generation of controlled crossing, several of the UMCA selections show significant improvements in yield and nut quality over existing cultivars. These selections may not only have potential for growers interested in highly productive orchards but could also serve as breeding parents for future generations of improved black walnut populations. However, this trial only included two years of nut quality data and only one year of yield data. For more reliable data on differences between selections and cultivars, additional years of observation will be necessary. In addition, replications of this trial in other environments with contrasting latitudes, elevations and soil types will aid in strengthening findings seen here. To this end, there is state-level support in Missouri to propagate these candidate releases and offer participant growers the ability to evaluate them further.

Figure 3.1 A necrotic eastern black walnut leaf infected with anthracnose.

Table 3.1 Twelve eastern black walnut selections ranked by their performance in 2007-2010. Values are expressed as percentile scores and a total percentile score in the right-most column.

Selection	Female Parent	Male Parent	% Kernel percentile	% Good percentile	Nut Wgt percentile	Diameter percentile	Yield Eff. percentile	Percentile Score
02.1055	Sparks 127	Football	0.992	0.94	0.551	0.775	1	4.258
02.1079	Sparks 127	Br. Nugget	0.86	0.936	0.535	0.631	0.777	3.739
02.1022	Sparks 127	Emma K	0.921	0.817	0.558	0.699	0.634	3.63
02.042	Br. Nugget	Daniel	0.73	0.905	0.837	0.919	0.437	3.829
02.461	Sparrow	Emma K	0.794	0.835	1	0.852	0.28	3.76
02.215	Daniel	Tomboy	0.759	0.85	0.966	0.928	0.315	3.819
2.1002	Sparks 147	Emma K	1	0.96	0.688	0.936	0.217	3.801
02.287	Sparks 127	Neel #1	0.778	0.96	0.726	0.805	0.388	3.657
02.1114	Sparrow	Emma K	0.963	0.905	0.704	0.919	0.272	3.764
02.463	Sparrow	Emma K	0.892	0.833	0.74	0.873	0.373	3.71
02.1053	Daniel	Football	0.931	1	0.752	0.839	0.119	3.641
02.530	Sparks 147	Emma K	0.955	0.96	0.759	0.886	0.083	3.642

Figure 3.2 Overhead (above) and field (below) photographs of the UMCA eastern black walnut selection trial population in 2020.

Figure 3.3 Five-stage scale of leaf emergence in eastern black walnut: 1—dormant buds, 2—swollen buds, 3—leaf emergence, 4—leaf expansion, 5—full leaf unfolding.

Figure 3.4 Field photos of high-yielding black walnut selections. Nut clusters (upper right) occur on the terminal ends of branches as well as along short spur branches (bottom right). Selections ‘02.1053’ (left) and ‘02.1114’ (upper right) produced large crops in 2021.

Table 3.2 The seven UMCA selections included in the yield performance and nut quality trial.

Selection	Female Parent	Male Parent	Live Ramets
2.1079	Sparks 127	Brown Nugget	4
2.1075	Sparrow	Emma K	5
2.1022	Sparks 127	Emma K	4
2.1114	Sparrow	Emma K	3
2.1055	Sparks 127	Football	4
2.1002	Sparks 147	Emma K	2
2.1053	Daniel	Football	5

Table 3.3 Yield and nut quality attributes of seven UMCA selections and 7 cultivars.

	No. of Trees	Yield (kg) per tree In-shell	Kernel[x]	No. of Nuts	Nut Mass (g) In-shell	Kernel[y]	Kernel %	Kernel Quality Color	Fill[z]
Year 2021									
UMCA 2.1002	2	3.36 (de)	1.19 (def)	181.11 (de)	14.83 (bcd)	5.27 (abcd)	0.35 (a)	2.80 (abc)	6.30 (abc)
UMCA 2.1022	3	3.45 (de)	1.06 (ef)	262.38 (cde)	10.53 (e)	3.23 (e)	0.31 (d)	3.24 (a)	4.20 (d)
UMCA 2.1053	3	19.79 (a)	6.66 (a)	1,089.39 (a)	16.26 (b)	5.50 (ab)	0.34 (abc)	1.88 (de)	6.67 (a)
UMCA 2.1055	3	13.43 (abc)	4.09 (bc)	602.03 (bcd)	15.87 (b)	4.85 (bcd)	0.31 (d)	2.94 (ab)	6.30 (ab)
UMCA 2.1075	4	15.97 (ab)	5.20 (ab)	726.68 (ab)	16.51 (b)	5.39 (abc)	0.33 (bc)	2.51 (bcd)	6.11 (abc)
UMCA 2.1114	3	11.99 (bc)	3.90 (bcd)	705.66 (abc)	15.60 (bc)	4.99 (bcd)	0.32 (cd)	2.62 (abc)	5.14 (bcd)
Brown Nugget	2	5.67 (cde)	1.55 (cdef)	312.50 (bcde)	13.77 (cd)	3.76 (e)	0.27 (e)	1.68 (e)	6.09 (cd)
Neel	3	10.15 (bcd)	2.84 (cde)	461.29 (bcde)	20.62 (a)	5.77 (a)	0.28 (e)	2.23 (cde)	6.44 (abc)
Sparks 147	2	0.30 (e)	0.10 (f)	15.00 (e)	13.28 (d)	4.72 (cd)	0.35 (ab)	1.65 (e)	6.10 (ab)
Sparrow	3	10.04 (bcd)	2.79 (cdef)	456.73 (bcde)	16.57 (b)	4.62 (d)	0.28 (e)	2.83 (abc)	5.67 (abc)

		Nut Mass (g) In-shell	Kernel[y]	Kernel %	Kernel Quality Color	Fill[z]	Quarters	Veins
Year 2017								
UMCA 2.1002	2	13.14 (de)	4.67 (cde)	0.36 (c)	2.22 (bcd)	1.39 (b)	2.17 (bcde)	2.28 (a)
UMCA 2.1005	5	16.01 (bc)	4.57 (de)	0.28 (ef)	3.01 (ab)	1.15 (c)	1.91 (cde)	1.04 (e)
UMCA 2.1022	3	11.26 (e)	3.90 (ef)	0.35 (c)	2.52 (bcd)	1.12 (c)	2.86 (abc)	1.81 (abc)
UMCA 2.1053	2	15.13 (cd)	5.43 (abc)	0.36 (bc)	2.09 (bcd)	1.06 (c)	1.71 (def)	1.65 (abc)
UMCA 2.1055	2	16.80 (abc)	5.47 (abc)	0.33 (d)	2.70 (abc)	1.13 (c)	3.15 (ab)	1.64 (abcd)
UMCA 2.1075	5	16.51 (abc)	5.72 (ab)	0.35 (c)	3.49 (a)	1.08 (c)	2.21 (bcde)	1.06 (de)
UMCA 2.1114	2	15.97 (bc)	6.12 (a)	0.38 (a)	3.20 (ab)	1.00 (c)	2.75 (abcd)	1.22 (cde)
Brown Nugget	4	12.87 (e)	3.81 (f)	0.30 (e)	2.41 (bcd)	1.09 (c)	1.83 (def)	1.79 (abc)
Daniel	3	17.96 (a)	5.88 (ab)	0.33 (d)	3.00 (ab)	1.03 (c)	0.87 (f)	1.97 (ab)
Football	3	16.57 (abc)	4.87 (cd)	0.29 (e)	1.66 (cd)	1.66 (a)	3.32 (a)	1.93 (ab)
Neel	4	17.47 (ab)	5.24 (bc)	0.30 (e)	2.14 (bcd)	1.00 (c)	2.23 (bcde)	1.44 (bcde)
Rupert	2	16.25 (abc)	4.33 (def)	0.27 (f)	2.29 (bcd)	1.05 (c)	1.66 (def)	1.25 (cde)
Sparks 147	2	13.18 (de)	5.00 (cd)	0.38 (ab)	1.45 (d)	1.05 (c)	1.30 (ef)	1.40 (bcde)
Sparrow	2	15.78 (bc)	4.75 (cd)	0.30 (e)	2.76 (abc)	1.00 (c)	2.97 (abc)	1.63 (abcd)

[x] Kernel yield calculated by multiplying in-shell yield by kernel percentage

[y] Kernel percentage = ratio of kernel mass to in-shell mass of good nuts

[z] Fill scored 1-5 in 2017 with 1 being highest fill, scored 3-7 with 7 being highest fill in 2021

Table 3.4 Pearson's correlations between nut quality, yield parameters, anthracnose susceptibility and phenology of 30 trees in the experimental planting including parental cultivars, selections and other progeny. Values approaching 1 are blue in color, values near zero are white and values approaching -1 are red in color. ***: p<0.001, **:p<0.01, *: p<0.05

	BB														
SL	-0.19	**SL**													
RD	0.28 **	0.89 ***	**RD**												
AnthS	-0.08	-0.07	-0.19	**AnthS**											
Yield (mass)	-0.02	0.21	0.19	-0.23	**Yield (mass)**										
Yield (nuts)	-0.07	0.15	0.12	-0.19	0.97 ***	**Yield (nuts)**									
NutWCC	0.00	0.46 *	0.45 *	-0.60 ***	0.42 *	0.37	**Nut WCC**								
Nut WSS	-0.17	0.450*	0.38 *	-0.56 **	0.35	0.29	0.83 ***	**Nut WSS**							
Nut LEE	0.10	0.387*	0.39 *	-0.15	0.15	0.10	0.19	0.32	**Nut LEE**						
Nut(mass)	0.00	0.50 **	0.49 **	-0.54 **	0.32	0.22	0.76 ***	0.79 ***	0.33	**Nut (mass)**					
Kernel(g)	-0.04	0.65 ***	0.61 ***	-0.54 **	0.43 *	0.34	0.59 ***	0.62 ***	0.51 **	0.87 ***	**Kernel (mass)**				
Kernel%	-0.08	0.45 *	0.42 *	-0.22	0.30	0.29	0.02	0.06	0.57 **	0.14	0.61 ***	**Kernel (%)**			
Quarters	0.08	0.09	0.07	-0.32	-0.29	-0.30	0.42 *	0.29	-0.12	0.26	0.02	-0.40 *	**Qs**		
Fill	0.14	0.39 *	0.42 *	-0.09	0.43 *	0.37 *	0.28	0.17	0.12	0.35	0.42 *	0.27	-0.08	**Fill**	
Veins	0.04	0.28	0.30	-0.07	0.12	0.18	0.15	0.13	-0.17	0.13	0.25	0.32	-0.10	0.14	**Veins**
Color	-0.09	-0.25	-0.30	0.12	-0.10	-0.13	0.02	0.06	0.05	0.06	0.04	0.03	-0.27	0.13	0.01

BB	Bud break date	WCC	Width Cheek to Cheek
SL	Season Length	WSS	Width Suture to Suture
RD	Ripening Date	LEE	Length Proximal End to Distal End
AnthS	Anthracnose Susceptibility	Qs	Quarters

Table 3.5 GCA values and LSD letters ($p < 0.1$) for 10 cultivars in the parental generation of the population. Traits without LSD letters showed no significance for GCA.

Cultivar	Nut Mass	Kernel Percentage	Kernel Mass	Kernel Color	Quarters	Kernel Fill	Kernel Veins
Br Nugget	-1.36 (d)	-0.72 (cd)	-0.38 (c)	-0.07 (bcd)	-0.16	0.04	0.01
Daniel	0.91 (a)	0.74 (abc)	0.32 (a)	-0.08 (bcd)	-0.50	0.05	0.01
Emma K	-0.32 (bc)	1.08 (ab)	0.11 (ab)	0.23 (ab)	0.02	-0.01	-0.01
Football	0.54 (ab)	-0.17 (bc)	0.08 (ab)	-0.35 (d)	0.32	-0.03	0.02
Neel	1.01 (a)	-0.43 (bcd)	0.16 (a)	-0.13 (cd)	0.07	-0.03	0.01
Rupert	0.3 (ab)	-1.96 (d)	-0.21 (bc)	0.18 (abc)	0.12	-0.02	-0.03
Sparks127	-0.16 (b)	-0.02 (bc)	-0.03 (abc)	0.37 (a)	0.30	-0.09	0.03
Sparks147	-1.12 (cd)	1.63 (a)	-0.04 (abc)	-0.32 (d)	-0.23	0.03	0.00
Sparrow	0.19 (ab)	-0.14 (bc)	0 (ab)	0.18 (abc)	0.05	0.06	-0.04

Table 3.6 SCA values and LSD letters ($p < 0.1$) for 17 crosses. Numbers correspond to cultivars Brown Nugget (1), Daniel (2), Emma K (3), Football (4), Neel (5), Rupert (6), Sparks 127 (7), Sparks 147 (8), Sparrow (9)

Cross	Kernel Mass	Kernel Percentage	Nut Mass	Quarters	Kernel Fill	Kernel Veins	Kernel Color
1x1	-0.1 (bcd)	0.21 (bcd)	0.02 (abc)	0.22 (a)	0.086	0.15 (bc)	-0.038
2x2	0.32 (ab)	0.12 (bcd)	0.12 (ab)	-0.06 (abc)	0.143	0.23 (ab)	0.119
2x3	0.05 (bcd)	-0.5 (bcd)	0.01 (abc)	-0.69 (bc)	-0.092	-0.22 (cd)	-0.185
2x4	0.07 (bcd)	1.97 (abc)	-0.08 (c)	-0.21 (abc)	0.111	0.05 (bcd)	-0.086
4x4	-0.02 (bcd)	-1.05 (cd)	0.03 (bc)	0.35 (ac)	-0.142	0.21 (abc)	-0.053
5x5	0.13 (abcd)	-0.26 (bcd)	0.04 (ab)	0.08 (abc)	0.14	-0.16 (bcd)	-0.036
6x6	-0.15 (b)	-1.36 (d)	0.01 (c)	0.16 (ac)	-0.028	-0.17 (bcd)	0.056
7x1	-0.29 (b)	-0.04 (bcd)	-0.15 (abc)	-0.09 (abc)	-0.071	0.03 (bcd)	0.008
7x3	-0.06 (bcd)	0.71 (abcd)	-0.1 (abc)	0.55 (ac)	-0.039	0.18 (abc)	0.1
7x4	0.14 (abcd)	-0.25 (bcd)	0.12 (ab)	0.17 (ac)	-0.017	-0.13 (bcd)	0.038
7x5	0.13 (abcd)	-0.47 (bcd)	0.1 (ab)	-0.01 (abc)	-0.449	0.61 (a)	0.016
8x3	-0.5 (d)	-1.4 (d)	-0.26 (d)	-0.06 (abc)	-0.095	0.25 (ab)	0.046
8x8	0.21 (abc)	2.58 (ab)	0.03 (ab)	-0.2 (abc)	0.135	-0.13 (bcd)	-0.093
9x1	-0.42 (c)	-2.04 (d)	-0.13 (ab)	-0.69 (b)	0.154	-0.12 (bcd)	0.036
9x3	0.76 (a)	3.68 (a)	0.29 (a)	0.25 (abc)	0.171	-0.37 (d)	0.139
9x6	-0.19 (b)	-1.8 (d)	0.03 (ab)	-0.09 (abc)	-0.071	-0.36 (d)	-0.035
9x9	-0.07 (bcd)	-0.08 (bcd)	-0.08 (abc)	0.31 (abc)	0.066	-0.06 (bcd)	-0.03

Table 3.7 Estimated broad and narrow sense heritability as well as additive and dominance variance of nut quality traits in 2017.

Trait	Heritability Broad Sense	Heritability Narrow Sense	Additive Variance	Dominance Variance
Nut Mass	0.404	0.373	1.689	0.144
Kernel Mass	0.504	0.231	0.149	0.176
Kernel Percent	0.69	0.32	3.627	4.177
Quarters	0.52	0.258	0.229	0.233
Kernel Fill	0.294	0.068	0.015	0.051
Kernel Color	0.343	0.281	0.174	0.038
Kernel Venation	0.427	0.036	0.009	0.095

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