CHESTNUT (*CASTANEA* spp.) CULTIVAR EVALUATION FOR COMMERCIAL CHESTNUT PRODUCTION IN HAMILTON COUNTY, TENNESSEE

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ABSTRACT

Chestnut cultivars were evaluated for their commercial applicability under the environmental conditions in Hamilton County, TN at 35°13' 45" N 85° 00' 03.97" W elevation 230 meters. In 2003 and 2004, 534 trees were planted, representing 64 different cultivars, varieties, and species. Twenty trees from each of 20 different cultivars were planted as five-tree plots in a randomized complete block design in four blocks of 100 trees each, amounting to 400 trees. The remaining 44 chestnut cultivars, varieties, and species served as a germplasm collection. These were planted in guard rows surrounding the four blocks in completely randomized, single-tree plots. In the analysis, we investigated our collection predominantly with the aim to: 1) discover the degree of acclimation of grower-recommended cultivars to southeastern Tennessee climatic conditions and 2) ascertain the cultivars' ability to survive in the area with *Cryphonectria parasitica* and other chestnut diseases and pests present. We hypothesized that some cultivars would perform well and could therefore be recommended to potential growers. Cultivars were primarily judged based on mean nut mass and total nut yield. Based on our results, recommendations for chestnut cultivars in Tennessee include 'Gideon', 'Nanking', 'Shing', 'Qing', 'Eaton', 'Eaton River', and 'Payne', which show promise in being generally abundant producers that also yield profitably sized nuts. Unlikely candidates based on mean nut mass and survivorship are 'Ford's Sweet', 'Little Giant', 'Heritage', 'Byron', 'Colossal', and 'Ford's Tall'.

DEDICATION

I would like to dedicate this thesis to my parents, Robert and Joyce Bundy, and to all chestnut researchers who have committed much time and effort for a better understanding of this worthy tree.

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LIST OF ABBREVIATIONS

Al, aluminum

Ca, calcium

CO_2, Carbon dioxide

COV, Coefficient of Variation

DNA, Deoxyribonucleic Acid

dsRNA, double-stranded RNA

ECM, Ectomycorrhizal fungi

HARC, Horticultural and Agriculture Research Center

INRA, Institut National de la Recherche Agronomique

K, potassium

Mg, magnesium

N, nitrogen

OA, oxalic acid

P, phosphorous

PPO, polyphenol oxidase

ppm, Parts Per Million

RAPD, Random Amplification of Polymorphic DNA

RNA, Ribonucleic Acid

RPP, Reconstructed Panmictic Population

siRNA, small interfering RNAs

sp, species singular

spp, species plural

SSR, Simple Sequence Repeats

TACF, The American Chestnut Foundation

TVA, Tennessee Valley Authority

US, United States

USDA, United States Department of Agriculture

vc, vegetative compatibility

CHAPTER I

INTRODUCTION

Species of chestnut have provided vital nutritional, cultural, and economical resources for many civilizations for thousands of years across East Asia, Europe, and eastern North America (Case, 2007). The fruit and the tree of the genus *Castanea* have had the dual role of being a staple food and providing wood, but the genus also was a keystone species in its ecosystem (Youngs, 2000). In some places, chestnut still serves as a semi-keystone species in areas where it is managed by farmers as an agro-ecosystem as opposed to conventionally managed forests (Aumeeruddy-Thomas et al., 2012). As a nut crop, cultivars of *Castanea* spp. have been planted far beyond the species' naturalized ranges (Conedera, 2004; Douglas, 2007). To ensure reliable, sufficient, and economically profitable harvests, it is important to identify which cultivars are most suited to particular climates through research and documentation of cultivars' characteristics. Hardiness, cold tolerance, disease-resistance, drought-resistance, superior taste, and peelability are among the features selected for modern commercial chestnut enterprises (Bounous, 1999). Consequently, it should be possible to grow chestnuts in Tennessee as a commercial nut crop. The goals of this study were to 1) assess the nut productivity of diverse *Castanea* cultivars, 2) to make recommendations to potential growers in Hamilton County, Tennessee, for commercial production, and 3) to assess whether chestnut cultivation could be a viable industry for this area.

Originally, we started with 64 different cultivars represented by 534 individual trees in 2003-2004 (Craddock et al., 2005). By 2012, we had 89 different cultivars represented by 563 trees. Some cultivars from the 2003-2004 tree plantings died, which we replaced with other cultivars by direct planting, or grafting onto rootstocks. I started working on the project in 2009. We planted our orchard at the Hugh Smith farm in Ooltewah, TN, at 35°13' 45" N 85° 00' 03.97 W elevation 230 meters. I evaluated our chestnut cultivars on nut mass, cultivar yield per tree, and general survivorship of cultivars that have survived from the beginning of the experiment in 2003-2004 to the present.

My analysis enables further understanding of factors that affect commercial chestnut production, such as pests, droughts, diseases, and limited access for marketing chestnuts. This study recommends some necessary

conditions and limitations in economically managing commercial chestnut orchards in this region, and suggests possible further research. The main objective was to provide cultivar suggestions for a successful commercial chestnut orchard in Tennessee. Although I have couched the cultivar comparison study in terms of simple comparisons between cultivars' nut size and productivity, the main issue has a wider scope. Principally, I assessed whether a commercial chestnut orchard could be a financially remunerative investment in Tennessee. This broader research question, based on my results, can tentatively be answered yes.

CHAPTER II

LITERATURE REVIEW

The genus *Castanea* Mill. in the Fagaceae (Nixon, 1997), is a relatively small genus whose range is found in southern Europe, eastern North America, northern Africa, Asia Minor, and eastern Asia. Three species are used for commercial production: *C. sativa* Mill. (European chestnut), *C. crenata* Sieb. and Zucc. (Japanese chestnut), and *C. mollissima* Bl. (Chinese chestnut; Hardin et al., 2001). Three species of chestnut occur in China: *C. mollissima* Bl., *C. senguinii* Dode (Seguin chinkapin), and *C. henryi* (Skan) Rehder & E.H. Wilson (Henry's chinkapin). Most of the distribution of *C. sativa* extends to the southern part of the European continent (Konstantinidis et al., 2008). *Castanea crenata* exists in Japan, and three species are found in North America: *C. dentata* Marsh (American chestnut), *C. pumila* Mill. (Allegheny chinkapin; Lang et al., 2007), and *C. ozarkensis* Ashe (Ozark chinkapin; Bost, 2011).

The term "chestnut" can refer to both the trees in the genus *Castanea* Mill. and the fruit of the trees in this genus. Although they are often mistaken for being chestnuts, the horsechestnut (*Aesculus hippocastanum* L.) and the buckeye (*Aesculus* spp.) are not chestnuts; nor is the water chestnut (*Eleocharis dulcis* Burm. f. Trin.), a grass-like sedge commonly used in Asian dishes (Bhagwandin, 2003). "Old chestnut" is also a phrase that describes a hackneyed cliché or tale. This expression originates from William Diamond's play, *The Broken Sword* (1816), in which a character, Captain Xavier, keeps repeating the same story about a cork tree with minor variations each time (Jack, 2005).

Chestnut ethnobotany

Chestnuts are arguably one of the most important nut crops in the temperate zone. Chinese chestnuts were economically important in China by the time of the Han dynasty (206 B.C.E.-220 C.E.; Qin and Feng, 2009). Archeological excavations in Japan have discovered large amounts of chestnut pericarps that date back to the early Jomon period (8000-7000 B.C.E.; Saito, 2009). Approximately eight species are included in *Castanea* that were naturally restricted to temperate regions of the Northern Hemisphere until anthropogenic influences spread them to

other regions (Taylor, 2004). The first unambiguous pollen data showing evidence of European chestnut trees spreading due to human activities date back to around 2100-2050 B.C.E. (Conedera et al., 2004). Since that time period, numerous cultivars have been developed in China, Japan, and Europe for *Castanea* and propagated through grafting. The International Code of Nomenclature for Cultivated Plants defines a cultivar as

> an assemblage of plants that (a) has been selected for a particular character or combination of characters, (b) is distinct, uniform, and stable in these characters, and (c), when propagated by appropriate means, retains those characters (Brickell et al., 2009).

Some of the indicators of anthropogenic spread of *Castanea sativa* are sudden rises in the slopes of pollen curves in palynological analyses (Bottema, 2003/2004). The first evidence of chestnut-human interaction is around 8600 B.C.E. where chestnut pollen presence became constant in northwest Syria (Yasuda et al., 2000). However, this is interpreted as being an indirect result of forest clearings intended for the primary purpose of cultivating olives, wheat, and barley (Yasuda et al., 2000). *Castanea sativa* likely extended its range into these cleared forests because of reduced competition. Evidence of the spread of chestnut due to human activity is found in regions in northern Greece, southern Bulgaria, and the Anatolian peninsula around 2100-2050 B.C.E (Bottema, 2003/2004). However, during this time period, it is difficult to distinguish between direct human cultivation and indirect influence such as forest clearings (Santos et al., 2000).

The Insubrian region was the first major fulcrum of *Castanea sativa* cultivation in Europe during the Roman Era. The introduction of the chestnut into the Insubrian Region, the region from the South Alpine Lakes to the Swiss-Italian border, radically changed human land use practices. Inhabitants stopped using fire to clear forests and began managing mountain forests as chestnut groves (Tinner et al., 1999). Conedera et al. suggested that *C. sativa* was a monoculture grown for its wood and coppice as well as its fruit after Roman introduction (Conedera et al., 2004).

The Romans spread *C. sativa* throughout the European continent to produce wooden barrels for preserving wine (Pereira-Lorenzo and Ramos-Cabrer, 2004), but chestnut as a food was not the main reason for the Romans' dissemination of the tree over Europe. The cultivation of *C. sativa* as a subsistence food primarily developed after the Roman period in association with the socio-economic systems of the medieval times. Pollen data and literary records confirm that chestnut also played a subsidiary role in ancient Greek civilization. The Ancient Greeks were an important cultivator of *C. sativa* for its wood and fruits, even though they never produced it on a large scale (Conedera et al., 2004).

In some areas of its cultivation, *C. sativa* was so crucial for human survival that some historians describe these cultures as "chestnut civilizations," such as in *La Civiltà del Castagno* by A. Gabrielli (1994). There are not many instances in which one can speak of a crop as having a civilization centered on it, so much that the disappearance of it leads to present-day nostalgia. A chestnut civilization is an appropriate characterization of some European regions in the past, such as in Galicia, Limousin, Tuscany, Auvergne, Trás-os-Montes, Corsica, and in the Cévennes (Pitte, 1987). J.R. Pitte, author of the book *Terres de Castanide* ("Land of Chestnuts"), proposed that a chestnut-centered culture, in Western Europe at least, was probably conceived in the Caucasus. Chestnuts were a particularly suitable crop for those areas that were unfit for growing cereals (Pitte, 1987).

An advantage of cultivating *C. sativa* trees was their low maintenance. Ariane Bruneton-Governatori in *Le Pain de Bois* (1984) estimated that chestnut orchards usually required about 3 to 8 days a year per hectare of trees. However, unless grafted cultivars were used, cultivating chestnuts required more time for seedlings to reach maturity than annual crops. Seedling chestnut trees generally start producing at about 15 years of age and take around 50 years to start bearing commercially optimal crops. Thus, in Cevennes there was a common expression that went, "Olivier de ton aïeul, Châtaignier de ton père, Mûrier à toi" ("Olive tree of your forefather, chestnut tree of your father, only the mulberry tree is yours"; Bruneton-Governatori, 1984).

In Japan, civilizations were in part built on *C. crenata*, as it furnished the material for railroad ties, telephone poles, houses, fuel, and furniture. It provided, in some cases, the primary nutrition for people. Chestnut stands were considered a secondary forest traditionally managed by communities and kept adjacent to villages. Sadly, almost all of these stands have disappeared in Japan due to economic development (Kitagawa et al., 2008).

In *Nihon Shoki*, the second oldest book of Japanese history (C.E. 720), the earliest record of the significance of *C. crenata* in Japanese culture is contained. In the *Manyoshu*, arguably the most revered compendium of Japanese poetry, a poem by Yamanoue-no-Okura relates the eating of chestnuts with nostalgic memories of childhood, a sentiment that many Japanese people share today. It is worthy of notice that the name *kachi-guri*, which refers to dried chestnuts in Japanese, can be broken down into the phonetically same cultural word "victory", indicating that chestnuts are considered an auspicious food (Kitagawa et al., 2008).

Despite its previous ranking as one of the most significant forest trees, generations in the United States have become more and more unfamiliar with the tree and the nut (Warmund, 2011). Although European chestnuts

have replaced American chestnuts in the U.S. market, there is no wood that has served as a substitute to *C. dentata* in versatility and durability (Buttrick, 1915).

Chestnut tree and nut characteristics

During late summer, differentiation occurs in flower buds on *Castanea*, which are produced distally on developing shoots above the maturing burrs. New shoots appear from these buds in the course of the subsequent spring with catkins, or aments, emerging along the shoot midway. Chestnut trees produce bisexual catkins and male staminate catkins. Usually, the first catkins producing pollen ten weeks after bud break are basal males. From the end of May to the middle of June, pollen is first discharged from the basal staminate catkins. A few days later, the females are pollen-receptive and remain so for one to two weeks. Then, bisexual catkins start to discharge pollen. Almost all chestnut trees are self-sterile and must have a pollenizer which differs from it. These trees should be within a 60-meter vicinity as chestnuts are principally wind-pollinated, although the flowers are visited by several pollen-feeding (as opposed to nectar-feeding) insects. Any two cultivars should be able to pollinate each other, unless one of them is male-sterile (Hasegawa et al., 2009).

In the chestnut, the pistillate flowers are located in the "burrs" or "burs" (involucres). If the flower is pollinated, they develop nuts (usually three nuts per burr, depending on the species). Each nut is derived from a single ovary. After fertilization, the growing embryo absorbs the endosperm and the nut thickens and increases in size of the cotyledons (McKay and Crane, 1939). A mature fruit of *Castanea* does not contain any endosperm. However, it is present in the early stages of nut formation (Jaynes, 1963). The chestnut is both a nut and a fruit, and the embryo is the edible portion (McKay and Crane, 1939).

There are usually three chestnuts per burr (involucre) (Hunt et al., 1998). Before the involucre dehisces, the plant increases production of the hormone ethylene (Payne et al., 1982). If all three nuts are formed, the center nut is flattened and the other two leveled on one side. One-nut-per-burr types, such as some Italian *marrone* varieties and *marrons*, will be globoid. Involucres can merge together, which will result in six to seven nuts per burr, but this is undesirable because the nuts will be deformed and flattened. Some cultivars fruit fused burrs less than others (Hunt et al., 1998).

Chestnut trees, being monoecious, bear male and female flowers on the same plant. Bisexual catkins tend to grow distally, near the shoot tip. The bisexual catkins generally have one or two female flowers at the base.

However, some commercial cultivars, such as Italian *marrone* types, bear astaminate catkins, meaning they produce no pollen and thus cannot serve as pollinizers (Pereira-Lorenzo et al., 2009).

Fortunately, most cultivars do produce pollen. There is some indication that the father-tree (the pollenizer) may have influence on the characteristics of the pollinated nut, a phenomenon known as metaxenia (Miller et al., 1996). This suggests that to facilitate the bearing of large nuts, the pollen from a tree with large nuts might impart a larger size to its recipient (Jaynes, 1963). Mckay and Crane (1939) reported that *C. crenata* produced distinctly different sizes of nuts as a result of using two kinds of pollen. When the pollen came from *C. mollissima* trees that yielded small nuts, the mean mass of the nuts produced was 18.77 g/nut; but, when pollen was supplied from *C. crenata* bearing large nuts, the mean mass of the nuts produced was 27.12 g/nut. This was a significant difference. This phenomenon also was observed when crosses were made between varieties of *C. sativa*, *C. crenata*, and *C. mollissima* (Mckay and Crane, 1939).

In a study conducted by Worthen and Woeste (2007) to determine the influence of pollen donor genotype on seed set and seed mass of *C. dentata*, the data showed that both the female and pollen donor contributed significantly to the variance in seed mass and seed set (Worthen and Woeste, 2007). The pollen parent may also play a role in the dormancy requirement, amount of carotene, and maturation time of the nut. Knowledge of a nut's dormancy period can be valuable in commercial nut production as nuts' values are enhanced if they store well and resist early germination (Jaynes, 1963). In a series of intraspecific and interspecific crosses of different species of *Castanea*, Jaynes (1963) observed that the male parent can affect nut dormancy. *Castanea pumila*, when used as a pollen parent, tended to decrease the dormancy period when crossed with other species. Therefore, chestnut growers in the southeastern United States may have a change in nut quality if their orchard trees are pollinated by the native chinkapin (Jaynes, 1963).

Tanaka and Kotobuki (1992) found that pollen parent influences peelability in some chestnut hybrids. When the hybrids were pollinated by *C. mollissima*, nuts were easy to peel, but when the pollen parent was *C. crenata*, nuts were difficult to peel (Tanaka and Kotobuki, 1992).

The apparent effect of the pollen parent on seed size may merely be hybrid vigor, or heterosis, expressed in the embryo of the seed. However, depending on the genotype of the parent, it is likely that heterosis will be represented differently in each cross (McKay, 1942). For example, in controlled crosses in European chestnut, Bolvanský and Mendel (1999) found the influence of male parent on seed set and fruit size to be only marginally

significant. However, pollen parents did significantly affect the length of pellicle intrusion (Bolvanský and Mendel, 1999). The most distinct differences were shown in precocity between families of interspecific hybrids and families of intraspecific hybrids. Interspecific hybrids were three times more precocious in flowering and fruiting than intraspecific hybrids (Bolvanský and Mendel, 1999).

It is reasonable to conclude that if the quality and size of the nut may be affected by the type of pollen used, then it should be possible to attain a more uniform size and quality of chestnut by planting an appropriate combination of pollinizers. Another implication of this is that it may be possible to prevent nuts with split shells since the pollen of certain cultivars determines the size of the nuts produced by other cultivars. This is desirable because chestnuts with split shells are more readily attacked by molds and thus spoil quickly (McKay and Crane, 1939). When choosing a pollinizer, it is of considerable importance that nut quality is not sacrificed for increased fruit set and productivity. Ideally, the pollinizers would shed pollen simultaneously with the blooming of the pollinated cultivars. An additional benefit would be that the pollinizers themselves produce nuts of marketable quality (Craddock et al., 1992).

Commercial chestnut production worldwide

Major chestnut-producing countries include China (FAO, 2000), Korea (Kim, 2006), Italy (Bounous, 1999), Japan (Ciesla, 2002), Spain (Pereira-Lorenzo et al., 2009), Portugal (Patrício et al., 2009), and France (Davidson, 2012). Countries that are becoming increasingly involved in chestnut production, although they contribute a small proportion of total world production, are Australia, New Zealand, Chile, Bolivia, Argentina (Ridley, 1999), and the United States (USDA, 2007). Worldwide chestnut production and value of production is shown in Table 1.

Table 1 Worldwide chestnut production[1]

Country	Annual harvest (t)	Value (US$/t)
China	1266510	$933.9
Korea	77524	$9661.1
Turkey	55100	$2357.4
Italy	50000	$1512.6
Bolivia	42801	$276.9
Japan	22100	$2870.5
Portugal	22000	$1495.2
Greece	14999	$2655.7
France[4]	10000[5]	n/a
USA[3]	4902	$2590.7
Australia[2]	1800	$6111.1

[1]Based on 2007 production except where indicated (FAOSTAT.FAO.org, 2012);
[2]Based on 2009 production. Data were unavailable at FAOSTAT.FAO.org. Data from Austnuts, Australia's nut directory (Austnuts.com.au/chestnuts);
[3]Based on 2010 production. The USDA does not separate imports and domestic production data (Geisler, 2010);
[4]Data for value unavailable at FAOSTAT.FAO.org; [5]Hennion, 2010.

China is the largest exporter and producer of chestnuts (Table 1). About one-third of its production (422,170 metric tons) is exported to Japan. Roughly three-hundred different cultivars are grown across the entire geographic range of China, but only approximately fifty are produced commercially (FAO, 2000). Although *C. mollissima* is the principle economic species, China has two other native species of chestnut trees: *C. henryi* Rehd et Wils (Chinese chinquapin or Henry's chestnut), which is harvested commercially, and *C. seguinii* Dode. (Chinese chinquapin or Seguin chinquapin), which is a commercially unimportant dwarf chestnut. China can produce chestnuts at a low cost, and thus sets the price in Asia (Qin and Feng, 2009). Common *C. mollissima* cultivars grown in China are 'Zunyu', 'Yanhong', 'Zipo', 'Duanci' (Wang et al., 2012), 'Dongmiwu wuhua', 'Yanchang', 'Chushuhong', and 'Zundali' (Ai et al., 2012).

Korea, the world's second largest producer, has been cultivating chestnut trees for more than 2000 years. About 37% of the annual 80,000 metric ton harvest per year is exported to Japan. Korea largely cultivates *C. crenata* and *C. crenata* x *C. mollissima* hybrids (Kim et al., 2005) and reports yields as high as 1.6 metric tons/ha with about 65 trees per hectare. Korea has the highest per capita consumption of chestnuts at 1.8 kg, followed by Japan (0.5 kg), Europe (0.45 kg), China (0.2 kg), and the U.S. (0.05 kg) (Olsen, 2000). Korea earns $80 million (US) per year on chestnut exports alone and $6.4 million for all other fruit exports combined (Ciesla, 2002). Korea may be able to command such high prices because about 37% of its harvest is exported to Japan (Vossen, 2000). Japanese importers are willing to pay high prices for chestnuts. Japanese manufacturers of chestnut delicacies, which are comparable to

gourmet chocolates, can sell their individually-wrapped gourmet sweet chestnuts for as high as $1 per nut (New, 1987).

In Korea, about 30% of the chestnuts consumed are fresh (Kim et al., 2005). The production of chestnuts is increasing each year in Korea, with consumption being far below production. Korea produces roughly 94,000 mt on the 80,000 ha devoted to chestnut production (Lee et al., 2011). Native Korean chestnuts have desirable nut characteristics such as peel-ability, sweetness, and a firm kernel. However, Korean chestnuts are highly variable in nut traits due to the fact that they are mostly grown from seedlings and not grafted. Common cultivars in Korea include 'Okkwang', 'Daebo', 'Tanzawa', 'Arima', 'Riheiguri', 'Tskuba', and 'Ginyose', which are reported to be cold-hardy and gall-wasp resistant. Since the introduction of the chestnut gall wasp to Korea in 1958, research has been focused on developing cold-tolerant and gall-wasp resistant cultivars (Kim et al., 2005).

Although Japan produces chestnuts and is the world's biggest chestnut consumer, it is the largest chestnut importer and its consumption is mostly based on imports (Ciesla, 2002). Some native *C. crenata* cultivars grown in Japan are 'Arima', 'Akachiu', 'Bonguri', 'Choubei', 'Yourou', 'Yakko', 'Togenashi', 'Tanabata', 'Shougatsu', 'Odai', and 'Katayama' (Nishio et al., 2011). 'Tzukuba', 'Tanzawa', and 'Ginyose' are amongst the most widely grown cultivars in Japan (Dini et al., 2012). Due to diseases such as chestnut blight, caused by *Cryphonectria parasitica*, and insect pest problems such as *Dryocosmus kuriphilus*, production is severely limited. Japan produces roughly 40,000 metric tons annually, but its major sources of chestnuts, in order of importance, are imports from China, Korea, and Italy (Ceisla, 2002).

Italy is another large producer and consumer. It is the largest chestnut producer in the European Community, and leads the world in producing processed chestnut products such as *marron glacé* (Bounous, 1999). Italian chestnuts are mostly harvested from "native" groves that have been managed for decades (Bounous, 2005). The prominent chestnut-producing regions in Italy, in order of their percent contribution to the national harvest, are Campania (45%), Calabria (20%), Lazio (14%), Toscana (7%), Piedmont (4%), Lombardy (2%), and Emilia-Romagna (2%). All other regions where chestnuts are grown contribute 4% (Pierrettori and Venzi, 2009). There are hundreds of named cultivars in Italy. Traditional Italian *C. sativa* cultivars include 'Tempuriva', 'Marrone di Borgovelino', 'Marrone di Val Suza', 'Marrone di Caprese Michelangelo', 'Fiorentino', 'Marrone di Chiusa di Pesio', and 'Marrone di Marradi' (Martín et al., 2010).

More than 80% of Italy's national chestnut production is consumed within 2-3 months after harvest. Half of that is eaten domestically and the other half is exported. The remaining 20% is either dried or processed in some way (Pierrettori and Venzi, 2009). Coppicing and high-canopied chestnut forests that provide saw-logs and serve multifunctional purposes, are also significant in Italy in addition to chestnut fruits (Craddock, 2007). An important element influencing the recent development of the Italian chestnut sector is the advanced technological progress in all three levels in the production process: in forest management practices, in harvesting technology, and in processing equipment (Pettenella, 2001).

Another important driving force is new marketing instruments that include certification of chestnut products' origins. For example, the *marrone* from Castel del Rio and the *marrone* from Mugello are registered under Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). Eight different chestnut flour specialties from the regions of Tuscany, Calabria, and Liguria are certified as Traditional Specialty Guaranteed (TSG) (Pettenella, 2001). Interest has been regenerated in traditional chestnut cultivars due to an expanding market for local foods with a traditional character that are perceived by consumers as being of higher quality. The development of certifiably authentic food products may be crucial for increasing the success of cultural food-influenced tourism (Stefano, 2012).

Although Italy has historically been one of the largest chestnut producers in Europe, its chestnut production and land devoted to chestnut trees has steadily decreased (Pierrettori and Venzi, 2009). The 50,000 mt production in 2007 in Italy (FAOSTAT, 2012) is a diminutive fraction of the 700,000 mt produced at the beginning of the 20th century (Pettenella, 2001). The combined blows of World War I, a Global Depression, World War II, chestnut blight, and ink disease have dealt devastating setbacks in commercial chestnut production in the 20th century (Bounous, 1999).

Like the Italian commercial chestnut enterprises, the French chestnut industry has diminished substantially as well, mostly owing to the conversion of former chestnut agricultural land to more profitable crops and urbanization. When wheat became a cheap and widely available crop in France around the 1760s, it began to compete with chestnuts in regions that had traditionally grown them (Fauve-Chamoux, 2012). The Renaissance was a veritable "Golden Age" of the chestnut in France, but by the middle of the 19th century, chestnut fruit production had severely declined (Pitte, 1987). Within little over a century's period of time, a drastic reduction of chestnut-

planted lands took place: 578,224 hectares were cultivated with chestnuts in 1852; and by 1975, only 32,000 hectares existed (Bruneton-Governatori, 1984).

A severe setback to chestnut production in France was a very harsh winter in 1709 which was so devastating it discouraged replanting. The Limoges' circular *L'Intendant* in 1738 reported that growers had not replanted even a twentieth of the chestnut trees that had frozen 29 years previously. There also were similarly ruinous winters in 1789 and 1870 (Fauve-Chamoux, 2012). Another blow dealt to chestnut production was the replacement of chestnut trees with mulberry trees in the Rhone valley, where the silk industry of Lyon wielded substantial influence. The cultivation of fast-growing mulberry trees, used as food for silkworms, promoted a cash economy as opposed to subsistence agriculture (Fauve-Chamoux, 2012).

The philosophy promoted by the Physiocrats also had a definite negative effect on the reputation of chestnuts in France. François Quesnay and Victor Riqueti de Mirabbeau, founders of the Physiocratic School, condemned chestnuts and alleged them to be a food that induced laziness. The French Enlightenment historian and writer François-Marie Arouet ("Voltaire", 1694-1778) supported the chestnut, asserting that it "is more nourishing and tastier than…barley or rye…which feeds so many people and is much better…than the bread ration given to soldiers" (Fauve-Chamoux, 2012). Over 200 years later, French ethno-historian A. Bruneton-Governatori also noted that chestnuts were nourishing and could easily provide 4,000 calories of energy per day.

Some pure *C. sativa* traditional French cultivars include 'Bouche Rouge', 'Verdale', 'Arizinca', 'Toumive', 'Belle Epine', 'Savoye', 'Châtaigne de Laguepie', 'Sardonne', 'Rouse de Nay', and 'Dorée de Lyon' (Mellano et al., 2012; Grau, 2003). The main Euro-Asian hybrid cultivars grown are 'Bouche de Bétizac' and 'Marigoule' (Hennion, 2010). In France, some of the best *marron* chestnuts, which are candied to make *marrons glacés*, are cultivated in Lyon. However, France's chestnut production has declined so much that most of their *marron glacés* are made from imported chestnuts from Italy (Davidson, 2012). Coppicing is still widely practiced in France as well as growing chestnut trees for their fruits (Aumeeruddy-Thomas et al., 2012). France consumes about 20,000 mt/y and produces about 11,000 mt. Chestnut producers in several provinces in France, such as Cévennes, Ardèche, and Corsica, certify their products with a French Label of Origin (AOC). To be certified, they must confirm their geographic origin and demonstrate the purity of their products (e.g. chestnut flour) by various biochemical tests. Within Europe, France is the largest chestnut importer, mostly buying from Italy, but also from Spain and Portugal (Alary et al., 2007).

Spain's production is mostly consumed locally, but it also produces chestnut lumber as opposed to just the fruits, with some cultivars being selected solely for timber production (Pereira-Lorenzo et al., 2009). There are four different chestnut orchard management schemes in Spain: 1) orchards for nut production, 2) coppice stands, 3) high-canopied forests for timber production, and 4) multifunctional grafted orchards for both nut and timber production with cultivars such as 'Loura', 'Garrida', and 'Parede' (Pereira-Lorenzo et al., 2009). Due to its high starch content, 'Garrida' is suited to many industrial purposes and is being used in European breeding programs (Pereira-Lorenzo et al., 2006). Despite its wide industrial uses, 'Garrida' appears to be mostly grown in Spain (Pereira-Lorenzo, 2010). 'Loura', a less common cultivar (Álvarez-Álvarez et al., 2006), and 'Parede', which is widely grown in Spain, are mostly restricted to Spain (Pereira-Lorenzo et al., 2003).

Chestnut trees in Portugal also are cultivated for multiple purposes, both for fruit and for timber. 'Judia' is the main cultivar in northwestern Trás-os-Montes, and is the most popular cultivar in Portugal. 'Longal' is the oldest cultivar in Portugal and is widespread; but 'Judia' and 'Martaínha' are almost as popular and are widely utilized. The introduction of New World food crops such as maize and potato into Portugal has reduced the importance of chestnuts and other traditional food crops. However, even as traditional food crops such as chestnuts are diminishing in some countries, like Portugal (Gomes-Laranjo et al., 2009), they are being introduced into others areas such as South America and Australia (Casey and Casey, 2009).

Chestnut growing for both nuts and timber is a new industry in the Southern Hemisphere, which includes Australia, New Zealand, Chile, Bolivia, and Argentina. Australia has even organized a grower's association. In these Southern Hemisphere countries, chestnut harvest lasts from March to April (Ridley, 1999). Chestnuts were brought to Australia by immigrants from China and Europe during the Gold Rush in the 1850s as well as after World War II. The chestnut trees in Australia are mainly *C. sativa* hybrids that are intermixed with the germplasm of *C. mollissima* and *C. crenata*. The most common cultivars grown in Australia are 'Red Spanish,' 'Buffalo Queen,' and 'Purton's Pride.' 'Buffalo Queen' does not peel well and thus has become less popular. When 'Colossal' was introduced from the United States, it did not fare well in the Australian environment. 'De Coppi Marrone,' named after Tony De Coppi, has become an industry standard after its introduction in the 1980s for its flavor and peel-ability. 'Marrone di Chiusa Pesio' from Northern Italy and 'Bouche de Betizac', a French Euro-Japanese cultivar, are also widely favored by growers and consumers (Casey and Casey, 2009).

Australia produced 1200 metric tons in 2009 which was valued at $5,400,000. There are more than 340 commercial chestnut growers. The largest chestnut-producing region is in northeast Victoria. Most orchardists use 6x6 m tree spacing. The industry uses seven levels of sizing grades that are based on what size holes the nuts fall through in the industry's grader (Casey and Casey, 2009).

Australia's national grower's cooperative, Chestnuts Australia Incorporated, levied a $.10 per kg tax on chestnuts to support research and development. Three commercial processors in Australia have the machinery to produce value-added products like flour, dried chestnuts, and freeze-dried chestnuts. These companies are the Australian Chestnut Processing Cooperative, Celebrate Health, and Australian Gourmet Chestnuts (Casey and Casey, 2009).

Perhaps inevitably, the Australian continent's previously blight-free advantage has come to a close. *Cryphonectria parasitica* was discovered at Eurobin in the Ovens Valley, Victoria in South Australia on September 8, 2010. Chestnut grower David McIntyre of Chestnuts Australia remarked that Australia had been trying to keep blight out for the last 50 years (Ainsworth, 2010[A]). Ovens Valley was declared a restricted area, which prohibited chestnut, oak, plant host material, equipment and packaging from being moved out of the area or off of any property (Herald Sun, 2010). Unfortunately, Australia's Department of Plant Industries plant manager Pat Sharkey reported outbreaks in other parts of the state of Victoria (Ainsworth, 2010[B]).

About 160,000 chestnut trees grow in Victoria and more than $10 million is earned each year from chestnut production in north east Victoria alone (Godwin, 2011). Southeast Australia's second largest chestnut producers, Tony and Carmelina Iaria, fortunately, were not hit by the blight (Woods, 2011). About 4400 chestnut trees were removed from the Ovens Valley soon after the outbreak. The Department of Plant Industries has determined that it is still possible for the disease to be eradicated. Commercial chestnut growers were to be reimbursed by the Federal Government for trees that are removed, as tree removal will result in significant profit losses. Assuming grafted cultivars are planted, chestnut producers who replant their trees will have a few years of delay before they can regain their investment (The Weekly Times, 2010). The majority of the chestnut industry in Australia is in the Ovens, Upper Kiewa, and Beechworth areas (Ainsworth, 2010[B]).

New Zealand, another Southern Hemispheric chestnut-growing area, had chestnuts introduced into the country by European immigrants in the 1800s. However, the plantings were not commercial. Instead, chestnuts were planted for their landscape value. Commercial production was not initiated until the 1980s. New Zealand has

somewhat of an advantage to growing chestnuts since it does not have *Cryphonectria parasitica*, *Dryocosmus kuriphilus*, or the *Curculio* spp. weevil (Klinac and Knowles, 2009).

Compared to New Zealand and Australia, Chile has the best competitive access to the off-season chestnut market in the northern hemisphere. Chile is the biggest nut exporter in the Southern Hemisphere and possesses many ideal conditions for growing chestnut trees: fertile soils, low risk of spring frosts, high humidity, and no reports of *Cryphonectria parasitica*. The average yield of chestnut orchards in Chile is 4,972 kg/ha (Joublan and Ríos, 2005).

Castanea was introduced to Chile in the 19th century by European settlers. Due to strict quarantine regulations, Chile does not have *C. parasitica*, but it does have *Phytophthora* spp. Although Chile has several areas that are suitable for growing chestnut, commercial production has been stymied by the lack of commercial cultivars as most growers use sexual propagation for orchard trees (Grau and France, 1998).

Commercial chestnut production in the United States

Assessing the actual production and sales in the United States chestnut market is problematic due to the fact that many small producers offer their harvest for sale directly to consumers (Craddock, 2009). On a nationwide scale, the Chinese chestnut is a novel tree crop for North America post-blight, but it is rapidly gaining popularity in Missouri and the Midwest (Warmund, 2011). In the United States east of the Rockies, plantings of chestnuts are predominantly open-pollinated seedlings and blight-tolerant Chinese chestnut or hybrids (Vossen, 2000). The West Coast and the Pacific Northwest states have some important advantages to growing chestnut trees: they are blight-free, do not have the major insect pests of chestnuts, and they have a suitable climate (Craddock, 2009).

Obviously, a nut grower would desire trees that possess blight resistance, and many growers would need those that are cold-hardy. *Castanea mollissima* generally meets these requirements. Conversely, the *C. sativa* or European chestnut is not as cold hardy and its blight susceptibility renders it impractical to cultivate east of the Rockies. Thus, European chestnuts are predominantly grown in the west. The small to medium-sized *C. crenata* tree (approximately 10.6 meters) also does not have the Chinese quality of cold-hardiness and it has poor quality fruit. However, desirable European and Japanese characteristics can often be acquired through hybridization (Hunt et al., 1998).

The chestnut market in the United States, post-blight, has been dominantly nested in ethnic markets, mostly European and Asian, but the incorporation of novel and ethnic cuisines into American gastronomy offers a

pragmatic possibility that this food will extend to a broader audience and gain new enthusiasts (Warmund, 2011). Within the United States, there is a diverse group of ethnicities and second generation immigrants, many of which come from cultures that valued the chestnut (Craddock, 2009). The ethnic diversity within the United States has provided a significant market base for chestnut purchases. As demand has increased, so have the number of chestnut farms (USDA, 2007).

Michigan is the leader for hectares devoted to chestnut production (USDA, 2007).The states with the highest area of chestnut farms that are of bearing age are Michigan (329 ha), California (135 ha), Oregon (134 ha), Florida (114 hehactares), and Ohio (112 ha) (USDA, 2007). Michigan has approximately 100 commercial chestnut growers, which include about 40 growers who have developed Chestnut Growers Inc., a cooperative that engages in the commercial promotion, sale, and distribution of value-added, processed chestnut products (Throne, 2007).

The United States' chestnut industry is in the process of formation. The production volume is low, operations are small-scale, and chestnuts are mostly harvested manually (Gold et al., 2005). Commercial chestnut growing in the United States has several challenges to overcome. These problems entail fungal diseases, pest insects, and a lack of organizations that handle, advertise, store, and market the nut. Therefore, even though there is an increasing demand from the public and steady, loyal buyers, it is difficult to meet those demands in the market (Payne et al., 1982). Still, considering the fact that the former range of the American chestnut extended over a broad geographic area, there is a high potential for commercial plantings across North America to be quite geographically diverse (Russell, 1987).

Genetic diversity of cultivars and rootstocks

In chestnut farming, there exists dual competing goals: to increase the productivity of crops and to conserve genetic diversity (Liu and Zhou, 1998). Local varieties or landraces that have served local economic and cultural needs in a region are often retained because of their well-characterized qualities and regional adaptation. Compounding the problem (and the urgency) of conservation of chestnut cultivar genetic diversity is the fact that newer and better cultivars are being planted that displace traditional cultivars. Many cultivars have been lost due to the upheavals and general abandonment of chestnut in the first half of the 20th century (Martin et al., 2010), which sometimes makes the true identity of a cultivar ambiguous (Martin et al., 2009). To achieve efficient implementation

of breeding programs and to introduce valuable traits into cultivars, clarification of the genetic diversity and relationships of wild and cultivated chestnuts is needed (Tanaka et al., 2005).

Cataloguing of traditional cultivars of chestnut trees is challenging because of various methods of denomination (Martin et al., 2010). Often, chestnut cultivars are named after their geographic origin, how they are prepared, their ripening period, or their appearance or morphology. Chestnut cultivars are also sometimes named after their harvest dates. For example, the early-ripening Spanish variety 'Sanmigueleña' is harvested on San Miguel's (Saint Michael's) day, which occurs at the end of September. In some cases, names are derived from a mixture of sources. For instance, in Andalusia, "Bravia" refers to trees that were produced from seeds, and "Pilonga" describes trees with large nuts that are easily peeled. Therefore, 'Bravia Pilonga', a population variety, is the name given to this variety because of these shared characteristics (Martin et al., 2009). Martin et al (2009) used the term "population variety" to refer to a group of cultivars that shared the same name but had different genetic profiles that did not match any other accessions because they are derived from seeds (i.e., not a clonal variety) (Martin et al., 2009).

Information about chestnut cultivars' true parentage is frequently not objectively documented (Martin et al., 2009). This is especially problematic in reviewing the historical literature on chestnuts since there are a variety of colloquial names (Conedera et al., 2004). Fortunately, DNA markers have been able to describe and classify chestnut cultivars in a way that is more accurate than morphological characterization (Martin et al., 2009). According to the UPOV (International Union for the Protection of New Varieties of Plants: descriptors proposed for chestnut), Biodiversity International, and the Food and Agriculture Organization (FAO), fruit tree species are based on morphological characters and their phenology. Recently, however, microsatellite or simple sequence repeats (SSR) markers have become the most common markers for analyzing genetic diversity due to the fact that they are distributed throughout the genome, are polymorphic, and are co-dominant (Martin et al., 2010). Some of this research has been conducted in southern Spain, or Andalusia, where chestnuts occupy more than 12,000 ha and are a significant agro-environmental crop. The results have shown that the distribution of genetic variability and morphological traits corresponds to the region of origin (Martin et al., 2009). Similar research has also been conducted in Italy (Botta et al., 2001; Boccacci et al., 2004; Martin et al., 2010), in the USA (Romero-Severson, 2010), and in China (Wang et al., 2008).

Using simple sequence repeats (SSRs), genetic diversity of ancient cultivar populations can be analyzed and their origins reconstructed. This method has been applied to ancient Spanish chestnut cultivar populations and results indicate that the populations differentiated into two primary origins of genetic variability (Pereira-Lorenzo et al., 2011). Numerous homonymies and synonymies were detected in Spanish cultivar populations, by Martin et al (2009). What was unexpected was the existence of two different morphological traits but the same SSR patterns such as in 'Pilonga de Parauta' and 'Pilonga de Jubrique'. These two cultivars were in fact morphologically different in the highly heritable characteristic of male catkin type, which indicates that they are two separate varieties of a cultivar (Martin et al., 2009).

Using SSR markers and morphological traits, it is also possible to assess how cultivars have become distributed in various geographic locations where chestnuts have traditionally been cultivated. The primary objective of one study by Pereira-Lorenzo et al (2011) was to determine the origin of cultivation and spread of chestnut cultivars in the Iberian Peninsula, Canary Islands, and the Azores. The study assessed the mechanisms that gave rise to chestnut cultivars. The results showed a strong genetic distinction between the northern and southern Iberian Peninsula. The main cultivar group was 'Longal', which accounted for 27% of the genotypes (Pereira-Lorenzo et al., 2011).

It appears that cultivar diversification in Portugal and Spain was a result of distinct genotypes being related via hybridization and mutation, regardless of whether they shared the same name or not. Genotypes were regarded as related when they have at least one allele per locus in common. When only one allele was different between genotypes, they were considered mutations. Within the 10 cultivar groups, 14% had only one allele different; thus, they were mutations of the original ortet and were subsequently planted in different orchards (Pereira-Lorenzo et al., 2011).

Almost all of the cultivars had mistaken identities, the reasons for which are numerous. When distinct genotypes were found in the same Reconstructed Panmictic Population (RPP) but shared the same name, this was indicative of intra-cultivar variability. In the 10 main groups of cultivars, 54% of 160 cultivar denominations were related either by hybridization, grafting, or mutation. These results provide evidence that the diversity of chestnut cultivars in the Iberian Peninsula is primarily the result of grafting. Microsatellites also confirm that groups of related cultivars arose through hybridization and mutation (Pereira-Lorenzo et al., 2011). There appears to be low variation among denominations corresponding to *Marrone*-type cultivars, which may be indicative of a common

origin. It is probable that when farmers exchanged grafting material, the cultivars acquired synonyms based on their geographic locations (Martin et al., 2010).

Likewise, the occurrence of clonality is contingent upon how important the cultivar is within a region. Intra-cultivar variability was predominantly explained by sexual propagation, as it is a common myth among growers in the Iberian Peninsula that the central nut of the burr reproduces the exact parental nut characteristics. In spite of, and perhaps because of, these human errors, cultivar domestication in the Iberian Peninsula shows a distinct geographic pattern. The structure appears to suggest that there are two main origins of genetic variability in cultivar groups in the Iberian Peninsula: the north and central area. Variability was highest in the north, but the central area was a significant source of origin of cultivar groups. Southern Spain, the Canary Islands, and the Azores have more recent plantations consisting of seedlings and grafts from the main cultivar origin areas. Though these areas are secondary origins of variability, it is worthwhile to consider that chestnuts have been grown in the Canary Islands at least since the 15th century, which is an adequate amount of time for superior cultivars to be developed (Pereira-Lorenzo et al., 2011).

As mentioned previously, 'Longal' is the main chestnut cultivar throughout the entire Iberian Peninsula. It is not only one of the most important cultivars in Spain, but its genes have been used to create new cultivars, almost as important, in different chestnut growing areas. Its progeny includes such cultivars as 'Bermella', 'Laga', 'Pilonga', 'Temprana', 'Pelona', 'Injerta', 'Verata', and 'Mondarina'. The origin of 'Longal' can be traced back to the region between the central Iberian Peninsula and Southern Galicia. From there, its scions and seedlings were distributed north and south (Pereira-Lorenzo et al., 2011).

Identifying and understanding the genotypes of cultivars is another approach that is being used to improve chestnut cultivars. Some of the aims of the Fagaceae Genomics Project are to identify cultivars' pedigrees and relationships, to characterize cultivars' germplasm, and to identify the genetic diversity and differentiation in native *Castanea*. Historically, this has been done using isozyme and RAPD analysis (Random Amplification of Polymorphic DNA). However, some of the shortcomings of these methods are that isozymes do not have adequate polymorphisms for precise differentiation and RAPDs have limitations where species hybridize freely (Romero-Severson, 2010). One of the newer methods being used for this project is the STRUCTURE software program. Jean-Romero-Severson at the University of Notre Dame, who is using this program for the Fagaceae Genomics Project, uses the majority rules process: if five cultivars are the same with 95% certainty, then they might be defined as

cultivar ‘X’ and the others are variants of cultivar ‘X’. This is important because once the identities are verified better crosses between cultivars can be made. Romero-Severson is using chloroplast tests because they are more robust than using the nuclear genome (Romero-Severson, 2010). Romero-Severson and Aldrich et al. (2003) have also developed microsatellite markers that are widely applicable to genetic studies of *Castanea* and other members of the Fagaceae (Aldrich et al., 2003).

Boccacci et al. (2004) showed that loci developed in oak (*Quercus* L.), a member of Fagaceae, allow for SSR analysis in *Castanea*, a taxonomically related species. This type of analysis has advantages over isozymes, as the latter are influenced more by physiological and environmental conditions. However, only a small portion (about 20%) of the loci isolated in oak can be practically used for the fingerprinting of chestnut (Boccacci et al., 2004).

Botta et al. (1999) also tested SSR markers isolated in *Quercus* on cultivars of *C. sativa* to evaluate their usefulness for genotyping chestnut. Their results support the possibility of using *Quercus* SSR markers for genotyping chestnut. It is intriguing to observe that the *marrone* type was easily distinguishable from other *C. sativa* cultivars. ‘Marigoule’ also had a distinct genotype (Botta et al., 1999).

As demand for particular varieties of chestnut has increased, so has the necessity of methods for reliable characterization and identification of the cultivars. Once this objective is met, it will be possible to certify that a cultivar is true-to-type. After a wide range of genotypes have been fingerprinted, the selection of loci for chestnut identification will be more optimal (Botta et al., 2001).

Traditionally, cultivars are identified by the observation of morphological characters. However, these expressions are heavily influenced by the environment, cultivation practices, and developmental factors. Serdar and Kurt (2011) found that leaf morphometric characteristics may be suitable for differentiation of chestnut cultivar genotypes. Leaf parameters such as lamina length and width, distance between lateral veins, leaf length, and leaf area were reliable features for distinguishing between chestnut genotypes. Nonetheless, they suggested that genetic diversity was better investigated using molecular markers (Serdar and Kurt, 2011).

DNA fingerprinting also is beginning to be used for rootstock as well as cultivar identification in order to protect plant varieties and patents and to avoid misidentification. Rootstocks are selected for their resistance to pest and disease stressors in specific environments, while cultivar scions are selected for fruit quality and productivity. Rootstock identification tools are useful where morphological traits such as leaf shape and appearance are not visible. Using this tool, growers can be more assured of the identity of their purchases (Liu et al., 2007).

Important diseases of chestnut trees

The most infamous diseases of chestnut are the chestnut blight, which is caused by the ascomycete fungus *Cryphonectria parasitica* (Murrill) Bar (Maynard et al., 2008), and ink disease, which is caused by the Oomycete *Phytophthora* spp. de Bary (Abreu et al., 1998). The North American and European species of *Castanea* are highly susceptible to both diseases. There is genetic resistance to both diseases in the East Asian *Castanea* species and there is an effective biocontrol for blight based on a virus (Maynard et al., 2008). Although some other species and genera (e.g. *Quercus*, *Castanopsis*, *Acer*, *Carya ovata*, and *Rhus typhina*) show some susceptibility, *C. parasitica* causes only superficial infections of the bark that uncommonly causes the death of the tree or even branches. In these species, *C. parasitica* infection usually causes perennially healing cankers (Cunnington, 2011).

The plant pathogen *C. parasitica* did not evolve with its hosts *C. dentata* and *C. sativa*, thus, it has rapidly spread as an epidemic in North America and in Europe. *Castanea mollissima*, which has evolved with the pathogen, does have resistance, as does *C. crenata*, although to a lesser extent (Anagnostakis, 1987). *Cryphonectria parasitica* was first observed in North America in 1904 at the New York Botanical Garden, in Europe in 1938 in Italy (Milgroom et al., 1996), in 1956 in France (Dutech et al., 2010), and in 1964 in Greece (Roane et al., 1986). *Cryphonectria parasitica* is thought to have been introduced into the US via nursery stock from Japan, as plant introduction records show that the vast majority of plant introductions at the time were from Japan and very few from China. Less is known about the introduction of chestnut blight into Europe (Milgroom et al., 1996).

Visible signs of infection on the chestnut tree include orange stromata which push through the epidermis (Anagnostakis, 1987). Perithecia forcibly discharge the ascospores. *Cryphonectria parasitica* conidia are frequently dispersed over short distances by splash from rain. Rain-splash typically forcefully discharges the ascospores which are spread by wind. Insects, birds, and mammals can also transport conidia. Infected grafting material can also be a source of transmission for the disease (Cunnington, 2011). Conidia can remain viable in the soil for years (Anagnostakis, 1987).

Both sexual and asexual reproduction occurs in *C. parasitica*. Selfing in *C. parasitica* is an intra-haploid mating process where progenies are produced from two mating types. Both mating types are detected in these progenies. Identical haploid genotypes can be the result of sexual as well as asexual reproduction. Dutech et al. (2010) found surprisingly low levels of recombination among *C. parasitica* despite the presence of compatible mating types when examining several *C. parasitica* isolates within forest chestnut coppices (Dutech et al., 2010).

Cryphonectria parasitica was once classified as *Endothia parasitica* Murr. Soon after its discovery, it was described in 1906 as *Diaphorthe parasitica* Murr., but it was renamed *E. parasitica* in 1912 (Roane et al., 1986). *Endothia* was changed to *Cryphonectria* because the two genera had differences in their stromatal morphology and ascospore septation. Stromata of *Cryphonectria* partially penetrate the bark, while those of *Endothia* are wide and erumpent (Myburg et al., 2004).

Cryphonectria parasitica appears as cankers on chestnut trees; and, if the trees have little or no resistance, a canker can girdle and eventually kill the tree (Davelos and Jarosz, 2004). The bark on a blighted canker may appear split, sunken, and slightly swollen (Roane et al., 1986). *Cryphonectria parasitica* does not reach the xylem of chestnut trees (Anagnostakis, 1987). Blight-resistant reactions in chestnut trees are associated with enlargement of the stem around the sight of lesion development. In contrast, blight susceptibility manifests itself as atrophied stem tissue (Roane et al., 1986). Blight interferes with phloem transport and the vascular cambium tissue. However, it cannot enter the root system and new sprouts may grow from the base of the girdled tree, but those too in turn will become infected (Davelos and Jarosz, 2004).

The pathogenicity of *C. parasitica* is related to how it uses tannins from chestnut as a nutrient source. Blight-susceptible chestnut extracts contain condensed tannins and hydrolysable tannins. Only high concentrations of hydrolyzable tannins are found in blight-resistant chestnuts. *Cryphonectria parasitica* produces an esterase that is able to degrade hydrolyzable tannins. High concentrations of hydrolyzable ellagitannins and gallotannins are associated with blight-resistant callus tissue; but this can be explained by the weak acidity of the hydrolysis products of ellagitannins and not by the toxicity of ellagitannins to *C. parasitica* (Roane et al., 1986).

The protein cryparin, a cell surface hydrophobin of *C. parasitica*, acts as a pathogenicity factor as it is requisite for completing the pathogen's disease cycle. After the fungus enters a wound in the bark, it colonizes the wound by forming a hyphal fan through which the fungus invades the healthy tissues. Beneath the outermost layer of the bark, a stroma is formed which consists of fungal hyphae and dead and dying tree tissues. The stromal pustules (pycnidia), which are asexual fruiting bodies, and perithecia, which are sexual fruiting bodies, develop within this stroma. Large numbers of spores are released when these fruiting bodies erupt through the bark (Kazmierczak et al., 2005).

Oxalate production appears to play a role in the pathogenicity of *C. parasitica* in that more virulent strains produce less oxalic acid. Oxalate binds with calcium found in the middle lamella between host cell walls in chestnut

bark, causing the bark to become more susceptible to hydrolysis. The process has been shown to be stimulated by calcium, which is found in the pectic materials in the middle lamella. Thus, the fact that virulent strains produce less oxalic acid seems counterintuitive. Continuation of studies examining the role of oxalate regulation in *C. parasitica* pathogenicity such as those conducted by Bennett and Hindal (1989) and Vannini et al. (1992) are needed to increase understanding of oxalate regulation (Bennett and Hindal, 1989).

Cryphonectria parasitica changes its growth rate in response to changes in temperature; and it can alter its growth rate rapidly. There is a liberal range of favorable temperatures in which *C. parasitica* can grow *in vitro* and *in vivo*. As long as temperatures are above freezing and below 38°C, some growth from *C. parasitica* will occur, the rate of which is determined by temperature. The temperature at which *C. parasitica* achieves the highest rate of germination of conidia is 25.7°C (Roane et al., 1986). The growth rate of *C. parasitica* increases as temperatures increases (Anagnostakis and Taylor, 1984).

Another environmental factor that can contribute to changing rates of stromatal production is a change in forest composition. Following a clear-cutting, stromata production increase at a rapid rate. Sporulation is much lower on suppressed chestnut stump sprouts which grow as an understory than on clear-cut trees. Also, *C. parasitica* mainly produces stromata on cracks of mature bark (Roane et al., 1986).

Chestnut stands in Europe and in some parts of North America have recovered due to biological control of *C. parasitica* through hypovirulence. This biological relationship is termed hypovirulence because it reduces the virulence of *C. parasitica* to its host, which is not the usual case with most fungal viruses. The majority of fungal viruses do not affect fungi's relationships with their hosts. The family *Hypoviridae* include viruses that cause hypovirulence (Liu and Milgroom, 2007). Of the four species of hypovirus that have been described, *Cryphonectria hypovirus I* (CHV-1) is most associated with biological control of *C. parasitica* and is prevalent in Europe, but is not found in North America except where it has been released for biological control (Milgroom and Cortesi, 2004).

Hypovirulence has not been successful in controlling *C. parasitica* in eastern North America (Milgroom and Cortesi, 2004). One notable exception is in Michigan. Native American hypovirulent strains were discovered in Michigan in 1976. These American hypovirulent strains have a different phenotype than European ones. Michigan has had success with hypovirulence because there are few vc types in the Michigan *C. parasitica* population (Anagnostakis, 1987). However, perceiving whether or not biological control of *C. parasitica* has succeeded or failed depends in part on the scale at which hypovirus control is intended to succeed: individual trees or tree

populations. Individual cankers that are treated with virus-infected isolates of *C. parasitica* generally heal and do not grow into lethal cankers. In contrast, mixed success has come from the spread of viruses to other cankers in fungal populations. Thus, even if a single canker or two is controlled, this is irrelevant on a population-scale if the tree succumbs to another canker which eventually girdles and kills the tree (Milgroom and Cortesi, 2004).

Vegetative incompatibility is attributed as being the primary limiting factor in the spread of hypovirulence in North America (Huber and Fulbright, 1992). Transmitting the hypovirulent European *C. parasitica* strain, called EP713, is not an easy task due to the fact that hypovirulence-associated viral genetic information is in the form of RNA rather than DNA. Furthermore, attempts to deliberately infect fungal strains with these viral genetic elements have not produced a form that was subsequently able to replicate (Nuss, 1992). The quandary for biological control of *C. parasitica* is that aggressive virus strains impede sporulation so much that the virus cannot be transmitted. In contrast, mild strains, although they may not inhibit sporulation, are not severe enough to prevent tree mortality. Therefore, for biological control to succeed, an intermediate level of virulence may be necessary (Milgroom and Cortesi, 2004).

To efficiently transmit viral dsRNA to a virulent strain of *C. parastica*, the strains must have the same vegetative compatibility groups (Nuss, 1992). Thus, populations that have a high diversity of vegetative compatibility (vc) types are less likely to proliferate in an area. As *C. parasitica* is native to East Asia, the greatest diversity of vc types have been observed there compared to Europe or North America (Liu and Milgroom, 2007). Genetic variation in *C. parasitica* is also higher in China than in North America. Surprisingly, however, DNA fingerprints are more variable in North America than in China. In China, subpopulations of *C. parasitica* are distinctly different in their structure than populations in Europe, North America, and Japan (Milgroom et al., 1996).

Despite the close geographic proximity of Japan to China, Japan and Europe and Japan and North America are more similar in their genetic structure of *C. parasitica* populations than Japan and China. One explanation of this genetic differentiation is that genetic drift occurred in populations of *C. parasitica* in Japan as there is low gene flow between Japan and China. Another is that different selective pressures exist in the two regions, which has genetically distinguished Japanese *C. parasitica* populations from Chinese *C. parasitica* populations. Also, *Cryphonectria parasitica* may be able to specialize to different *Castanea* species. However, restricted gene flow and genetic drift are the more likely explanations, as selection influencing multiple unlinked loci would be highly irregular statistically (Milgroom et al., 1996).

Fortunately for chestnut growers, vc incompatibility is not an absolute barrier to viral transmission. Over a long enough time and frequent encounters, transmission might occur even in high vc-type diversities. Also, it is not impossible that vertical transmission of hypoviruses be maintained via conidia during clonal reproduction (Liu and Milgroom, 2007). Cytoplasmic-transmissible double-stranded RNA (dsRNA) is also, at least indirectly, responsible for hypovirulence in certain populations of *C. parasitica*. For example, cytoplasmic-transmissible dsRNA is responsible for hypovirulence in populations of *C. parasitica* in Michigan; but, hypovirulence can also be caused by genetic components other than dsRNA. Hypovirulence could be influenced by mutated, dysfunctional mitochondria (Fulbright et al., 1992).

Symptoms of reduced virulence of *C. parasitica* include a reduction of enzymatic activities, pigmentation, and sporulation, as well as an alteration of colony morphology. During hyphal anastomosis, these dsRNAs with the hypovirulent phenotype can be transmitted to non-hypovirulent, dsRNA-free strains. Thus, in principle, it should be possible to apply a hypovirulent strain to a canker caused by a virulent strain, which should result in the canker healing (Choi et al., 1991). However, an obstacle of putting this approach into practice is RNA-silencing (Segers et al., 2007).

In fungi, RNA silencing may act as a defense mechanism against invasive viruses. In plants, RNA silencing has been shown to provide a defense against viruses. RNA silencing, or RNA-mediated suppression of gene expression (termed quelling in fungi and post-transcriptional gene silencing in plants), has a common feature with different organisms in that it processes dsRNA into small interfering RNAs (siRNAs) by using endonucleases called Dicers. Dicer-like genes play a role in protecting against virus infection in fungi. The RNA-silencing pathway in *C. parasitica* functions as an unspecialized antiviral defense mechanism which is not specific for hypovirus infections (Segers et al., 2007).

Most viruses of plants and animals have an extracellular phase in their replication cycle; but viruses of fungi uniformly lack this characteristic. Viruses in fungi are intracellularly transmitted through one of two processes: (1) transmission through asexual spores or (2) cytoplasmic exchange during hyphal anastomosis. Mycovirus transmission between different strains of the same fungal species is often governed by a genetic nonself recognition system regulated by vegetative incompatibility (*vic*). Vegetative incompatibility is sometimes visibly evident when conspecific, but genetically distinct, strains grow together in the same medium but form a barrage along the zone of contact. It can also be examined by letting conspecific strains form a heterokaryon. If the

heterokaryons exhibit no growth, abnormal morphology, or slow growth, then they are likely incompatible (Choi et al., 2012).

At least six known *vic* loci, with two alleles at each locus, control the *vic* system in *C. parasitica*. Hyphae of *C. parasitica* strains will freely fuse and support virus transmission if the strains contain the same alleles at all *vic* loci. Cell death will occur and virus transmission will be restricted if one or more alleles at the *vic* loci are different (Choi et al., 2012).

Although the exact mechanism of how hypovirus reduces the virulence of *C. parasitica* towards its host is not entirely understood, enzyme inhibition of *C. parasitica* upon its host may be implicated in the relationship. In culture, hypovirulent strains of *C. parasitica* produce less oxalic acid (OA) than virulent strains. Due to its reducing ability to chelate divalent cations, OA may function as an inhibitor on polyphenoloxidase (PPO) and other enzymatic activities. PPO levels tend to rise in plant tissues following pathogenic infection or mechanical injury. However, PPO activity has not been shown to increase in *C. sativa* following infection with either virulent or hypovirulent strains of *C. parasitica* compared with healthy plant tissue. Data obtained from *in vivo* experiments showed no difference in OA production in virulent or hypovirulent strains. Thus, if virulent strains of *C. parasitica* do mechanistically function as an enzyme inhibitor, it is apparently not by producing oxalic acid or by inhibiting PPO production in *Castanea*. Therefore, virulent strains must inhibit some other enzymes in *Castanea* (Vannini et al., 1992).

A plant pathogen which may come to be even more significant than chestnut blight is ink-disease. Some chestnut species are highly susceptible to root rots caused by the Oomycete *Phytophthora* spp. de Bary (Abreu et al., 1998). Ink disease on *Castanea* was first discovered in Portugal in 1838 (Vettraino et al., 2001), and in the USA in 1896 (Rhoades et al., 2003). Since then, two species have spread over Europe and N. America: *P. cambivora* Petri and *P. cinnamomi* Rands. Vettraino et al. (2001) showed that *P. cambivora* is the most aggressive species (Vettraino et al., 2001). In Italy, *P. cambivora* is the most common form of ink disease (Biocca and Motta, 1993). *P. cinnamomi* first came to N. America from Southeast Asia most likely on potted plants (James, 2011).

Castanea sativa and *C. dentata* are highly susceptible to *Phytophthora* root rot, but *C. crenata* and *C. mollissima* are not. Interspecific hybrid cultivars (*C. crenata* x *C. sativa*) show some degree of resistance as do *Castanea dentata* x *C. mollissima* and *C. dentata* x *C. crenata* (Ramos Guedes-Lafargue and Salesses, 1998).Trees infected with the disease typically begin declining with hot weather in the spring. One of the indicative symptoms is

that the water-conducting vessels become streaked black or brown (Vannini and Vettraino, 2001). Another initial symptom is yellowing of the leaves and partial canopy defoliation (Oliveira et al., 1998). Necroses at the base of the stems are symptomatic of the latest stages of the disease's development (Vettraino et al., 2001). Leaf chlorosis, also symptomatic of the disease, signifies a lower nutrient concentration in plant tissues. This may indicate an interference with nutrient uptake or metabolism (Pires et al., 1998).

Ink disease has been shown to significantly decrease leaf and inflorescence production in chestnut. It not only affects the quantity of nut production, but the quality of the nuts. Unlike *C. parasitica*, *Phytophthora* spp. damages the roots, which impacts the quantity of litterfall produced and thus affects N, P, K, Ca, and Mg availability in the soil (Pires et al., 1998). Leaf analyses of chestnut trees severely affected by ink disease show that Ca content is highly reduced in the leaves (Portela et al., 1998). Researchers from the Institute of Plant Pathogens at the University of Naples Federico II have developed a technique that measures electrolyte leakage from tissue of chestnut leaves that enables screening for resistance in a large number of chestnut cultivars in a short period of time. This avoids the difficulties of inoculation which is often time-consuming and dependent upon test conditions in the site (Cristinzio, 1993).

Different soils may be less affected by *Phytophthora* than others. Soils that are fertile, and have a low concentration of cations, and have a high phosphorous (P) content, may still have the pathogen's presence but fail to produce serious disease (Abreu et al., 1993). Elevated quantities of exchangeable cations in the soil tend to promote higher biological activity and greater resistance to ink-disease. Higher amounts of organic material and the presence of microorganisms, which may be antagonistic to *Phytophthora* spp., may reduce trees' susceptibility to the pathogen (Martins et al., 1998).

Drought conditions can also destabilize chestnut tree growth, reducing its vigor and making it more susceptible to *Phytophthora* ink disease (Gomes-Laranjo et al., 2005). Dry periods during summer followed by soil waterlogging in wet seasons are environmental factors that are conducive to the expression of the disease (Abreu et al., 1998). A site property that seems to be relevant in predisposing chestnut trees to ink disease is the amount of radiation the soil receives. South-facing slopes with higher temperatures frequently show severely affected chestnut groves as they provide conditions for a water deficit in the summer and accelerate soil organic matter decomposition. The severity of ink disease tends to decrease with higher soil fertility, deeper rooting systems, low intensity tillage and fertilization, and lime applications (Portela et al., 1998). The formation of sporangia and

zoospore discharge required for the development and reproduction of *Phytophthora* are promoted by prolonged soil saturation and poor soil aeration. Although *Phytophthora* can be found on a range of soil textures, such conditions are common in soils with high clay content (Rhoades et al., 2003).

Controlling the effects of this soil-borne pathogen is mainly based on the breeding and selection of different cultivars and the application of chemicals (Portela et al., 1998). For chestnut growers on a small scale, there are treatment options. However, these treatments are only temporary and must be applied repeatedly or the trees will die. Dr. Joe James recommends, in order of relative efficacy, potassium phosphite, urea phosphite or aluminum phosphite. If applied early, they can have a greater positive effect as a preventative (James, 2011). Fungicides used to control ink disease include propamocarb, cymoxanil, metalaxyl, and phosethyl Al. Such treatments are expensive and labor intensive. They also must be carefully applied so that beneficial fungi, which may possibly be antagonistic to *Phytophthora* spp., are not negatively affected (Abreu et al., 1993). A current method for treating *P. cinnamomi* is the use of mono- and di-potassium salts of phosphoric acid (phosphite is the general term). Perkins et al. (2012) examined chestnut ectomycorrhizae formation after administering phosphite and found that chestnuts which were given routine phosphite treatments exhibited fewer ectomycorrhizal root tips than those which were not given phosphite (Perkins et al., 2012).

In chestnut groves in Italy where *Phytophthora* spp. has been recovered from soils, the isolation frequency was much higher around trees showing symptoms of ink disease than from around healthy-looking trees. *Phytophthora cambivora* is usually associated with diseased trees and not recovered from soils around chestnut trees that are only slightly affected or non-symptomatic. Vettraino et al. (2001) suggested that *P. cambivora* is only able to disseminate from infected roots and newly infect for a restricted period of the year under favorable climatic conditions. However, the species *P. citricola* Sawada is able to infect during unfavorable conditions and can cause disease on chestnut, albeit to a lesser degree. From their data they inferred that *P. cactorum* (Lebert & Cohn) J. Schröt. and *P. citricola* infect chestnut to a lesser degree (Vettraino et al., 2001).

Attempts are being made to identify the genes for *Phytophthora* resistance in *Castanea*. Between and within chestnut species and hybrids, susceptibility to ink disease varies (Ramos Guedes-Lafargue and Salesses, 1998). The Institute National de la Recherche Agronomique (INRA) in Bordeaux has an ongoing interspecific hybridization program, one of the objectives of which is to create *Phytophthora*-resistant cultivars and rootstocks. Their research shows that resistance is not a recessive trait. They advise that a cultivar be screened at the adult stage

before a final selection is made (Salesses et al., 1993). Other assessments of resistance to ink disease have sought out possible compounds, such as proanthocyanidins, in resistant species that may be antagonistic to ink disease. Chestnut bark is relatively high in ellagitannins, which are polyphenols, but low in proanthocyanidins, which are condensed tannins (Abreu et al., 1998). Proanthocyanidins supposedly have a toxic effect on fungi. Different levels of proanthocyanidin content are observed in chestnut species. In one study by Abreu et al. (1998), *C. mollissima*, despite having low levels of proanthocyanidin, showed the highest resistance to *P. cinnamomi*. Therefore, *C. mollissima* resistance may be more attributable to other antifungal metabolites (Abreu et al., 1998).

Ectomycorrhizal (ECM) fungi on tree roots have been demonstrated to form a protective barrier against *Phytophthora* infection. Rhoades et al. (2003) explored the relationship between soil compaction, soil moisture, and ECM on *C. dentata* seedlings and found that soil compaction had a significant effect on aboveground biomass (Rhoades et al., 2003). However, Rhoades et al. (2003) found no significant relation between ECM and root necrosis or *Phytophthora* occurrence on *C. dentata* seedlings. Thus, the protection against *P. cinnamomi* that ECM fungi provide may necessitate denser networks of fungal mycelium as well as the presence of certain ECM species. The implications of such findings for American chestnut restoration are that reintroduction to American forests may only include a small portion of the American chestnut's former range (Rhoades et al., 2003).

However, a longer contact time with chestnut roots and mycorrhizae in the seedling stage may prove useful for establishing chestnut trees. Martins (2010) found that higher survival was achieved in mycorrhizal plants than non-mycorrhizal plants when the plants were in contact with soils affected by *Phytophthora cinnamomi*, but survival depended on the time of mycorrhization. The longer the time between the formation of mycorrhizae and contact with the pathogen, the greater the resistance of mycorrhizal plants and their increased probability of survival (Martins, 2010). Branzanti et al. (1999) also found that chestnut seedlings with ectomycorrhizal fungi showed less negative effect from ink disease pathogens which had been inoculated with the seedlings (Branzanti et al., 1999).

In research regarding *C. dentata* ink disease susceptibility, a screening technique developed by Dr. Joe James of the American Chestnut Foundation has led to the discovery of two closely-linked genes for resistance to *Phytophthora* in the *C. mollissima* cultivar 'Nanking'. By comparing these genes to a similar region of the peach genome, Dr. Albert Abbott at Clemson University was able to isolate a known gene for *Phytophthora* resistance. The implication of this research is that it is likely that the gene can be cloned onto another species or cultivar to see if it confers resistance to *Phytophthora*. This is being attempted with *C. dentata* (Sisco, 2011). Other research done

in Australia is being conducted to determine if some cultivars are resistant to *Phytophthora* root rot. One trial identified the cultivar 'Menzies' to be the least susceptible (Casey and Casey, 2009).

The main deterrent of using ectomycorrhizae to control root-borne diseases, despite their great potential, is that their efficacy is unpredictable, rendering them unreliable to use on a large scale (Pinnix, 2005). The best preventative measures relied on by nurseries and growers are quarantines of plant materials, varietal tolerance, and isolation to avert introduction and the dispersal of root rot (James, 2011). As there may be negative environmental impacts from the use of chemical applications to control ink disease, an integrated approach to chestnut ink diseases, involving the use of ink-resistant cultivars, well-drained planting sites, and early mycorrhizal inoculation of seedlings, could lead to better control of the disease (Branzanti, 1999).

Important pests of chestnut trees

The most noxious insect problems associated with chestnut are the chestnut weevils, *Curculio caryatrypes* (Boheman), *C. sayi* (Gyllenhall), and *C. elephas* (Gyllenhall). The North American weevil species are host-specific to *Castanea* (Keesey and Barrett, 2008). *Curculio elephas* and *C. sikkimensis* (Heller), found in Europe, attack chestnuts and acorns (*Quercus* spp.) (Desouhant, 1998). They lay eggs inside the nuts and the larvae feed on the cotyledon. Adult *C. sayi* also feed on chestnut catkins. If they are not controlled, their populations can destroy an orchard's production (Keesey and Barrett, 2008).

In the United States, *C. caryatrypes* emerges from the end of July to the beginning of September. Adult *Curculio sayi* start to emerge in mid-May to early June. There is also a second adult emergence period during late August to mid-October. The mature larvae burrow to a depth between 7.5 and 15 cm in the soil. *Curculio sayi* remain underground for about 19 months and have a life cycle of 20-21 months. Dryer conditions make it difficult for weevils to move through the soil. Circle traps and pyramid traps are commonly used to control weevil populations (Keesey and Barrett, 2008).

In Europe, the chestnut weevil *Curculio elephas* mates and lays eggs after the adults emerge from the ground in late August through September. The larvae feed on the chestnut fruit and then burrow into the ground to overwinter. The adult female lays eggs inside the chestnut fruit, but oviposition causes no visible damage to the husk. Several females can oviposit in the same fruit (Desouhant, 1998). In *C. elephas*, some individuals have a prolonged larval diapause, meaning that adult emergences are spread over 3 to 4 years. Drought conditions disrupt

the sex ratio of the population, making it switch from female-biased before emergence to male-biased after emergence (Menu, 1993).

The codling moth *Cydia pomonella* (L.) is an economically significant pest in Europe. Control of the insect's population is still largely based on insecticide applications. Fortunately, mating disruption, an environmentally safe control, is an available alternative method. This method uses dispensers to administer pheromones that disrupt mating behaviors by leading the insects down a "false trail" (Angeli et al., 2007).

Experiments by Debouzie et al (1996) suggest that the moth *Cydia splendana* interferes with larval development of *Curculio elephas*. *Cydia splendana* also has larval development in the chestnut fruit. Moth larvae of *Cydia splendana* in the fruit inhibit *Curculio elephas* weevil egg-laying. However, *Curculio elephas* weevil presence does not inhibit *Cydia splendana* egg-laying. Fortunately for the chestnut grower, this competition results in a reduction in the number of chestnuts infested by both insects (Debouzie et al., 1996).

There is also promise of a biological control of *C. elephas* by means of the entomopathogenic fungus *Beuvaria bassiana* (Balsamo) Vuill. In a three-year trial conducted by Paparatti and Speranza (1999), *C. elephas* larvae that were treated with *B. bassiana* had a significantly higher mortality rate than untreated *C. elephas* larvae (Paparatti and Speranza, 1999). *Curculio elephas* and other chestnut weevils are generally negated by prompt harvesting, washing, and storing, which avoid the cost of spray programs (Ciesla, 2011).

Certain bur types on *Castanea* may provide protection against beetles such as *C. elephas*. Female beetles may access the cupule surface for oviposition on cultivars with less dense spines on their burs more easily. The density of the spines on 'Torcion' is higher than on 'Lüina', although the spines are shorter. Sieber et al (2007) found that the fruits of the variety 'Lüina' had a higher infestation average than 'Torcion' (Sieber et al., 2007). Cultivars with long spine lengths of the burrs are postulated to provide natural protection against the oviposition of larvae of *Cydia* and *Curculio*. 'Longal' from the Iberian Peninsula is an example of a cultivar with long spine lengths, while 'Rapada' from Galicia has short spine lengths (Pereira-Lorenzo et al., 2009).

Another chestnut insect pest found in Japan (but not yet reported in the United States) is *Curculio sikkimensis* Heller. *Curculio sikkimensis* is widely distributed throughout Japan. The females oviposit into chestnuts and acorns during autumn and the larvae develop inside the fruits. When the larvae are mature, they leave the fruits and burrow into the soil where they begin diapause. Adults emerge from the soil after approximately one month.

However, when larvae enter prolonged diapause, the adults' emergence is distributed over several years (Higaki and Toyama, 2012).

Due to an extended diapause in the larval stage, *C. sikkimensis* has a multi-year life-cycle. Temperature controls its prolonged diapause. Its larval diapause is completed by recurring exposure to chilling and warming conditions. When *C. sikkimensis* larvae are alternately exposed to high and moderately high temperature and then chilled, this has been shown to result in different levels of diapause-completing effects (Higaki and Toyama, 2012).

The initiation and development of prolonged diapause are governed by genetic as well as environmental factors. In *C. sikkimensis*, low temperatures (≈5°C) can stimulate the completion of diapause. However, cycles of warm temperatures followed by cool temperatures are more effective for the cessation of prolonged diapause than exposing the larvae to cool temperatures alone. Higaki and Toyama (2012) suggested that larvae in extended diapause that are subjected to high temperatures (≈25°C) undergo extended diapause (Higaki and Toyama, 2012).

As *Curculio elephas* and *Curculio sikkimensis* inflict significant economic damage on chestnut production in Europe, Northern Africa, and the Near East, it is fortunate that the pest has not been introduced to the United States yet (Ciesla, 2011). However, the exotic invasive Asian gall wasp (*Dryocosmus kuriphilus* Yasumatsu) poses a threat to chestnut orchards in the eastern United States. The Asian gall wasp was introduced in 1974 on nursery stock and scion-wood, which is the primary method of infestation (Payne, 1976). *Dryocosmus kuriphilus* was introduced from China to Japan in 1941 and spread rapidly throughout the country after World War II (Moriya, 2003). *Dryocosmus kuriphilus* was reported on the Korean Peninsula in 1958 and Japan in the same year (Aebi et al., 2007); and was first detected in Italy in 2002 (Quacchia et al., 2010).

The chestnut gall wasp invades the vegetative buds and interrupts the shoot growth by making the plant form galls. This pest can devastate yields 50-75% (Payne et al., 1982). Once infestation has occurred, no method of chemical control can practically be used to manage *D. kuriphilus* as the thick wall of the gall protects the larva (Moriya et al., 2003). *Dryocosmus kuriphilus* generally has an annual generation rate, and larvae overwinter inside the chestnut tree buds (Cooper and Rieske-Kinney, 2010). *Dryocosmus kuriphilus* is both univoltine (having only one generation per year) and thelytokous (exhibiting a form of complete parthenogenesis where unfertilized eggs develop into diploid females) (Zhu et al., 2007). In the spring when the buds begin to leaf out, the larvae develop rapidly and convert the bud into a swollen pinkish-colored gall. Galls are abnormal growths that are induced on the host plant in which immature wasps develop. Galls protect the developing wasps from predators, pathogens, and

grazing from insect herbivores. These growths also act as nutritive sinks for the developing wasps (Cooper and Rieske-Kinney, 2010). Before pupating, the larvae feed for 20-30 days within the galls. The adult wasps completely emerge within 3 weeks. *Dryocosmus kuriphilus* spreads through infested wood or by the flight of the adults in May and June. The adults are only present two to three weeks. Chestnut growers may reduce infestation by pruning gall-infested branches and then burning them (Payne, 1976).

Dryocosmus kuriphilus is a serious threat to chestnut industries in Korea and Japan. Although there are resistant trees that have been bred, another strain of the wasp has already evolved that attacks the resistant trees. Late-ripening varieties of *C. crenata* are generally more resistant than others; and *C. mollissima* has relatively low resistance (Payne, 1976). However, the plant tissue of the host forms the galls, therefore, the phenotype of the host can influence gall initiation and growth (Cooper and Rieske-Kinney, 2010).

Although *D. kuriphilus* is native to China, it is still one of the most important pests on chestnut in China. The Anhui Agricultural University in Hefei, China analyzed different cultivars of *Castanea mollissima* to ascertain their resistance to gall wasp. In order of the most resistant, the cultivars 'Chushugong', 'Mifengqiu', and 'Ershuizao' were found to have immunity (Ding et al., 2004). A breeding program in Japan was formed in 1952 to develop gall-wasp resistant cultivars which resulted in the cultivars 'Ishizuchi', 'Tanzawa', and 'Tsukuba' being released. Since then, more resistant cultivars have been bred which include 'Kunimi' and 'Shiho' (Saito, 2009). As of 2005, the most common cultivars grown in Japan are 'Ishizuchi', 'Tsukuba', 'Tanzawa', and 'Ginyose' (Saito, 2009). Gall-wasp-resistant cultivars grown in Korea include 'Arima', 'Ginyose', 'Ibuki', 'Tanzawa', and 'Tskuba' (Park et al., 1981).

Another approach to combat gall-wasp has been to release the natural predator of the gall-wasp, *Torymus sinensis* Kamijo, from China. This parasitoid has spread throughout Japan since its introduction in the 1980s and the effort has been successful (Quacchia et al., 2010). Two other biological controls, *Torymus tubicola* Osten Sacken and *T. advenus* Osten Sacken, which are parasites of *D. kuriphilus*, have also been used to combat chestnut gall wasp (Payne, 1976). Although selecting resistant cultivars is valuable complementary research, the natural enemy *T. sinensis* is the most effective control of *D. kuriphilus* (Quacchia et al., 2010).

Torymus sinensis was introduced to Japan in 1975. At first their spread was gradual, less than 1 km/year, but it soon accelerated to an average rate of 60 km/year (Moriya et al., 2003). When *T. sinensis* was released in Japan, it hybridized with the indigenous *T. beneficus*. Fortunately, the hybrids are not sterile and interbreed

successfully with *T. sinensis* (Yara, 2006). In 1977, three species of gall wasp parasitoids were introduced in Byron, GA from Japan. Since then, gall wasp populations have dropped in Georgia and incidences of infestation have been apparently contained by the parasitoids' introduction and plant quarantines. The southern edge of the range of *D. kuriphilus* in the eastern USA in Peach County, GA has not spread since 1983 (Rieske-Kinney, 2007).

Torymus sinensis was also released in Italy in 2003 and has been released every spring since 2005 in open fields. This release program is funded by the Regione Piemonte local government (Quacchia et al., 2010). The release program has set aside an isolated orchard specifically intended for breeding *T. sinensis* (Quacchia et al., 2010). Gall wasp is a serious concern in Italy. In orchards that are highly infested, a loss of 40-70% in fruit production has been observed. In 2008, the minister of agricultural politics declared a state of natural calamity due to gall wasp infestation. What is especially disturbing is that the insect has colonized the Apennine Mountains through active adult flight, not through propagation material (Graziosi and Santi, 2008).

The introduction of *T. sinensis* is one of the most well-known cases of successful biocontrol (Moriya et al., 2003). However, the interaction between exotic pests and their hosts and native and non-native natural enemies has rarely been observed and studied. This scenario has the potential to create new communities (Cooper and Rieske-Kinney, 2011). For example, *Ormyrus labotus* Walker (Hymenoptera: Ormyridae) is a native parasitoid of oak-galling cynipids in N. America that has been shown to parasitize *D. kuriphilus. Torymus sinensis* also serves as a host for the parasitoid *O. labotus*. Thus, *O. labotus* is a hyperparasitoid of *T. sinensis* and may potentially suppress *T. sinensis* populations (Cooper and Rieske-Kinney, 2011).

Ormyrus labotus exploits multiple hosts and most likely requires other cynipid host(s) throughout a portion of summer months and winter months. Although other gall-formers exist in North America, including *Amphibolips confluenta* Harr. (oak apple gall wasp), *Callirhytis cornigera* Osten Sacken (horned oak gall), and *Eriophys amelanchieri* Stebbins (serviceberry gall mite), *T. sinensis* has not been observed to parasitize non-*D. kuriphilus* galls. Thus, *T. sinensis* is a generalist parasitoid of cynipid gall wasp in China, but acts as a specialist outside of its native range in Japan and North America where *D. kuriphilus* galls are abundant (Cooper and Rieske-Kinney, 2011).

Torymus sinensis and *D. kuriphilus* have co-evolved together. Therefore, *T. sinensis* is likely better adapted to parasitize *D. kuriphilus* than *O. labotus*. Also, parasitism of *O. labotus* may be limited to galls of a certain size as *T. sinensis* has a longer ovipositor than *O. labotus*. For both *O. labotus* and *T. sinensis*, parasitism on *D. kuriphilus*

is negatively correlated with gall size before *D. kuriphilus* emergence in June/July. Also, the thicker the schlerenchyma layer in the galls is, the less the galls are visited by parasitoids (Cooper and Rieske-Kinney, 2010).

Another threat to chestnut orchards are ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Unfortunately, the eastern United States has the most favorable environmental conditions conducive to ambrosia beetle (Schiefer and Bright, 2004). The wood-boring beetles of the family Scolytidae and the ambrosia fungi they carry in special pharyngeal sacs called mycangia, are highly evolved examples of animal-fungus mutualism. The ambrosia fungi are exclusively fed upon by the beetle larvae; and the adults transport the fungi from tree to tree. The female beetle burrows into trees, which are commonly physiologically-stressed deciduous hosts, and introduces the fungus as she lays her eggs. The beetles do not actually digest the masticated wood. Instead, they use the wood as a medium to grow their fungi. This fungal biomass, termed ambrosia, sporulates all over the walls of the brood galleries. Typically, beetles will carry specific species of ambrosia fungi in their mycangia, though their larvae may feed on other fungi that may be present in the insect tunnels (Kendrick, 2000).

In evolutionary terms, the easiest strategy for wood-boring Scolytid beetles to secure their host habitat is by opportunistically breeding in dying or dead tissue as opposed to killing part of or the entire host plan, for this tactic avoids having to struggle against constitutive host resistance. The pathogenic fungi may have compounds that suppress the tree's resistance mechanisms' activation (Kühnholz et al., 2003).

The ambrosia shothole borer, *Xyleborus dispar* F. was first reported in the US in 1817 and first reported utilizing *Castanea* as a host species in 1912. It has the potential to become a serious limiting factor in successful chestnut orchard establishment (Bhagwandin, 1993). Although this species has not been reported in Tennessee, other species in the genus *Xyleborus* have been (Atkinson et al., 1990). *Xyleborus dispar* is the only Scolytidae species to exhibit a true diapause. Frass tailings, wilting of newly emerged bud growth, and brittle branches or trunks are signs of ambrosia beetle attack (Bhagwandin, 1993).

The total *Xyleborus* spp. life cycle from egg to adult for both males and females is 10-11 weeks. Adult females might leave the colony to find a new host or continue to excavate their existing host. The males are flightless and spend their entire lives in the galleries in which they were born mating with their sisters. There are typically two generations per year and brood development depends on temperature. At ambient temperatures over 10°C beetles are able to bore through bark at a faster rate (Bhawandin, 1993).

Ambrosia beetles usually have two distinct seasonal flights: an early spring and summer flight. Flight distances have been reported at the rate of 11.27 km in four hours, which translates to 24-32 km/day for several days. Although ambrosia beetle attack is primarily limited to physiologically compromised hosts, they will attack perfectly healthy trees if their population is in epidemic or outbreak proportions. *Castanea* seems to be a preferred host for *Xyleborus* spp. (Bhagwandin, 1993).

One of the most economically important exotic ambrosia beetles in N. America is *Xylosandrus germanus* Blandford, which is of East Asian origin. First detected in New York in 1932, it has since become widespread across the US. It has been introduced in Europe since 1951 (Ranger et al., 2010).

In the adult phase, *X. germanus* overwinters within the wood of its host. Overwintering adult females can begin flight activity in Tennessee as early as March. The males are flightless. Although *X. germanus* prefers deciduous hosts, it is a generalist with an extensive host range. The fungus within the ambrosia beetle is usually introduced to weakened trees. Enervated trees secrete greater concentrations of volatile organic compounds such as methanol, acetone, acetaldehyde, ethane, ethylene, and ethanol. A diverse array of stressors can increase production of these volatiles. These stress-induced volatiles constitute important host-location cues for ambrosia beetle (Ranger et al., 2010). Trees under attack by ambrosia beetles might also trigger a feedback loop, as the trees might release monoterpenes through the flow of resin, thereby further attracting other beetles including those of different species (Miller and Rabaglia, 2009). *Xylosandrus germanus* is primarily attracted to ethanol. Thus, traps that are ethanol-baited are commonly used for ambrosia beetles. Also, injecting trees with stress-related volatiles, particularly ethanol, has been demonstrated to reliably induce attacks by natural populations of *X. germanus* (Ranger et al., 2010).

Using trunk injections to facilitate beetle attacks could become a useful strategy to mass trap ambrosia beetles. For example, in plum orchards, sentinel/sacrificial apple trees baited with traps dispensing the host volatile benzaldehyde and the pheromone grandisoic acid attract the plum curculio (*Conotrachelus nenuphar* Herbst) away from the plum trees, which reduces the necessity for insecticide application (Ranger et al., 2010). However, the magnitude of attacks probably depends on population density, which may vary between different locations and years. An olfactory-based trap tree scheme may prove useful for reducing ambrosia beetle attacks on *Castanea* and other important nursery stock as well (Ranger et al., 2010). The flight activity of ambrosia beetles are commonly

monitored with ethanol-baited traps prior to implementing insecticide control programs in horticultural tree nurseries (Miller and Rabaglia, 2009).

Diseases post-harvest

Chestnuts are highly perishable. They have high moisture content and cannot be stored like many other edible nuts. Post-harvest spoilage is most often caused by fungi and the larval development of insects. The insect damage most commonly associated with chestnut spoilage in Italy is from the pests *Cydia splendana* and *Curculio elephas*. Fungal development is often concomitant with larval development, and fungal infections often begin in the larval settlements of insects. Most nuts also have spores on them before picking (Jermini et al., 2006).

Microbial decay after harvest and during storage is one of the major limiting factors in producing quality chestnuts. An estimated annual economic loss of $30,000 due to postharvest decay was reported in 2007 for Michigan, which represents more than 25% of its total crop and 5,300 kg of fresh chestnuts (Donis-González et al., 2009a). In the United States, Italy, France, Australia, Chile and other countries, *Penicillium* sp., *Aspergillus* sp., *Sclerotina pseudotuberosa*, *Fusarium* sp., *Acrospeira mirabilis*, and *Phomopsis castanea* are among the molds most often identified. Amongst these, *Sclerotina pseudotuberosa* Rehm (synonym *Ciboria batschiana* Zopf.) is a true plant pathogen, meaning that it is capable of initiating infection prior to harvest. *Phomopsis castanea* Sacc., an endophyte in chestnuts, has been most prevalently reported in Australia and Chile (Donis-González et al., 2009a).

Experimental studies conducted on the storability of chestnuts show spoilage ranging from 5-10% of the nuts after 1 month to 15-60% in 7 months at 2ºC. Nuts that harbor noxious mycoflora can potentially spread them into processed goods through the market. Since some of the above-mentioned species are capable of producing mycotoxins, it is important to minimize weevil infestation and, consequentially, post-harvest spoilage (Payne et al., 1982).

Mycotoxins are toxic fungal secondary metabolites, which can be present in spoiled chestnut fruits (Donis-González et al., 2009b). Donis-González et al. (2009b) found that the cultivar ‘Colossal’ surpassed the federal maximum levels set for mycotoxins after but not prior to 90 days of storage. ‘Everfresh’, a *C. mollissima* selection from Michigan State University Experiment Station, so named for its extremely long shelf life (Rogers Reserve, 2012), never approached the maximum level and had the lowest concentrations of mycotoxins compared to ‘Colossal’ and ‘Eaton’. ‘Eaton’ mycotoxin levels varied depending on the mycotoxin. ‘Eaton’ was similar to

‘Colossal’ ochratoxin concentration levels after 90 days but was similar to ‘Everfresh’ deoxynivalenol and zearalenone levels after 90 days of storage (Donis-González et al., 2009b).

While some endophytic molds cause symptoms of disease after the fruit has fallen, the fungal mycelia degrade the cotyledons mostly while chestnuts are stored. In the beginning phases of infection, it is not easy to distinguish slightly moldy or parasitized nuts from intact ones without processing or consuming them. Therefore, a variety of post-harvest nut treatment methods have been developed, the most common of which are cold baths and warm baths (Jermini et al., 2006).

A cold bath is an immersion in cold water (15°C), followed by stirring for 15 minutes and removal of floating debris and floating chestnuts. Then, the chestnuts are left immersed in 15°C water for 8 days with the water changed every 2 days. After 8 days the water is drained off. A warm bath is an immersion in warm water (45-48°C) followed by stirring for 45 minutes and removal of floating debris and floating chestnuts. Then, the water is drained off and the chestnuts are immediately cooled in running water (12°C) for 12 hours, after which the water is drained off. The warm bath is now the standard technique because it can inhibit larval development without diminishing nut quality (Jermini et al., 2006).

Other methods of prolonging the postharvest life of chestnuts include Controlled Atmospheric Storage (CAS) (Jermini et al., 2006). In CAS, O_2 is reduced and/or CO_2 is elevated to levels different from those in the air. CAS implies the maintenance of precisely specified levels of O_2 and CO_2. The reduction of O_2 concentrations around fresh fruits reduces their respiration rate. When CO_2 levels are concomitantly increased, a shift from aerobic to anaerobic respiration occurs in fresh fruits when the room is kept below a minimum of about 1 to 2% O_2 (Zagory and Kader, 1989).

Washington et al. (1997) found that chestnuts stored in a controlled atmosphere (2.5% carbon dioxide/2.5% oxygen/95% nitrogen) at 0°C have a low percentage (9%) of kernel rotting after 70-172 days of storage than nuts not stored in a controlled atmosphere (CA) that were kept 0°C. Nuts stored in CA as previously described that were subsequently flushed with carbon monoxide (6% CO) at 0°C had an even lower incidence (6.1%) of kernel rotting after 70-172 days of storage (Washington et al., 1997).

As a corrective measure, sorting through spoiled nuts can be done through floatation in water. However, nuts invaded by weevils will not necessarily float, and chestnuts may be infected absent any visible spoilage (Payne et al., 1982). Avoiding contamination is easier than decontaminating products, but, investigations in postharvest

treatments can potentially prevent excessive spoilage and contamination of stored chestnuts (Donis-González et al., 2009a).

In one evaluation conducted in Michigan on the efficacy of postharvest treatments for reduction of mold and decay in fresh chestnuts, 0.92-ppm ozone solution (Aqua Air Technologies®) and hydrogen dioxide (1:100 dilution of Storox™) were determined to be best suited to protect against postharvest chestnut mold and kernel decay. The sanitizer ozone was not effective in reducing mold severity, but it did reduce the incidence of decayed kernels. Wax postharvest treatments did not reduce weight loss of chestnuts during storage (Donis-González and Fulbright, 2009). Storox™ (2700-ppm hydrogen peroxide + 200-ppm peracetic acid) and Flint® (0.15 trifloxystrobin) significantly suppressed the incidence and severity of shell mold and kernel decay. Overall, Storox™ and 0.92-ppm ozone solution (Aqua Air Techologies®) were most recommended to protect against postharvest chestnut mold (Donis-González et al., 2009 b).

Contamination does not only occur in orchards. The peeling process itself can strongly influence contamination of peeled chestnuts. The skin separator (brushes) and sorting belt have been identified as key points for mesophilic aerobic bacteria and yeast transmission in Michigan chestnut samples (Donis-González and Fulbright, 2009). The place from which chestnuts are harvested also influences chestnut spoilage. Donis-González and Fulbright (2009) found that chestnuts harvested from orchard floors were significantly more contaminated by post-harvest spoilage than those harvested directly from the tree (Donis-González and Fulbright, 2009).

Avoiding and preventing contamination as well as microbial reduction strategies will need to be further developed and monitored in order to determine their most efficient application.

Grafting and vegetative propagation of chestnut trees

Domestication is defined as "the outcome of a selection process that leads to increased adaptation of plant and animals to cultivation (Pereira-Lorenzo et al., 2011)." Clonality is implied when vegetative reproduction replaces sexual reproduction, which is one of the most important attributes of crop evolution. Most domesticated chestnut orchards are propagated through grafting (Pereira-Lorenzo et al., 2011).

Clonal propagation is a trustworthy method to conserve the fittest genotypes in a domesticated population, which is why it has been so widely used in Europe. Over half of the main Spanish cultivars (57%-58) are clonally propagated (Pereira-Lorenzo et al., 1990). In Italy, grafted cultivars exhibit low intra-cultivar (within the same

cultivar) variability (Fineschi et al., 1994). By crossing different cultivars together in the same orchard, diversity can be maintained or increased, which is desirable because with clonality the number of genotypes are reduced (Pereira-Lorenzo et al., 2011). It must be noted, however, that there are a number of ways to clonaly propagate (Macdonald, 1990).

The major methods of vegetative propagation used for woody plants are grafting and budding, cuttings from stems and roots, layering, and micropropagation (Macdonald, 1990). Certain methods of grafting may enhance the success of graft compatibility. The whip and tongue graft, widely used in the nursery trade, has a high success rate for chestnut trees as the grafted plants generally have vigorous growth. If the rootstock's and scion's diameters match exactly, the graft heals quickly and the union is very strong. The scionwood must be cut from the mother plant during full dormancy and stored in sealed plastic bags. As soon as the rootstock's dormancy is complete and it begins its vegetation is when grafting begins (Craddock and Bassi, 1993).

Most commercial chestnut growers use cultivars that have been selected by individual growers and the industry. Propagating cultivars is commonly done by whip-and-tongue grafting on 1-and 2-yr-old seedlings; and mature rootstocks can be top-worked by the same method or by bark grafting. Unfortunately, graft incompatibility is often an issue, especially on Chinese grafts (Payne et al., 1982). Delayed graft incompatibility has also been observed with *C. crenata* x *C. sativa* clones grafted onto *C. crenata* seedlings (Craddock and Bassi, 1993). To combat graft incompatibility, fruit breeders generally recommend grafting onto seedlings that are open-pollinated descendants of the desired cultivar (Payne et al., 1982).

Santamour (1989) has suggested that graft incompatibility may be minimized by grafting onto isozyme-typed seedling rootstocks. This has been demonstrated in red maple (*Acer rubrum* L.) (Santamour, 1989) and *C. mollissima* (Santamour, 1988). There are four known isozyme groups in *Castanea* spp., types A, B, AB, and BC. Among interspecific hybrids, which have very high isozyme heterogeneity, graft incompatibility has been an issue. When a rootstock's isozyme phenotype matches that of the scion, a greater percentage of vascular continuity has been observed (Craddock and Pellegrino, 1992). However, Huang et al. (1994) did not find similar isoperoxidase isozymes to have any effect on graft union success (Huang et al., 1994).

A grafted chestnut tree's orchard performance can also be predicted based on how the graft union appears. Good appearance of a graft union is indicated by uniform diameter between the rootstock and scion without swelling. In a study conducted by Craddock and Bassi (1999), *Phytophthora*-resistant rootstocks were evaluated

with four Italian ‘Marrone’ cultivars. Grafts onto seedlings of ‘Marrone’ cultivars and wild-type *C. sativa* seedlings had excellent graft unions and high percentages of successful grafts. *Phytophthora*-resistant rootstocks are valuable because many historically important chestnut cultivars are susceptible. *Phytophthora*-resistant rootstocks may ensure their survival. The *Phytophthora*-resistant hybrid cultivars ‘Marigoule’ and ‘Marsol’ had a high percentage of successful takes (unions) with ‘Marrone’ cultivar scion grafts, and ‘Marsol’ had high quality graft unions (Craddock and Bassi, 1999).

It is difficult to isolate the specific reasons for graft failure in clonal propagation of *Castanea* (Oraguzie et al., 1999). In a study carried out to determine whether cultivars’ origins and relationships affect graft failure within New Zealand cultivar selections, Oraguzie et al. (1999) found that cultivars with *C. mollissima*-like gene regions did not have any graft failure. However, *C. sativa* and *C. crenata* hybrids grafted poorly regardless of whether they were used as rootstocks or scions (Oraguzie et al., 1999). Huang et al. (1994b) also found Japanese hybrids to have a significantly lower success rate (<50%), irrespective of rootstocks (Huang et al., 1994b).

In China, millions of chestnut trees are successfully grafted each year. However, graft incompatibility still exists in China, and experiments conducted there suggest that the major cause of graft failure is the distinctive structure of the *Castanea* stem, which is fluted or grooved. Huang et al. (1994b) proposed that graft failure is partially due to rootstocks not being adequately characterized (Huang et al., 1994b).

Cloning is a strategy used to preserve the fittest genotypes in a population; but it has the potential to dilute genetic diversity. The reduction in diversity produced by grafting can be mitigated by the use of seedlings and mutations, the latter of which are often propagated by grafting. Grafting does not, however, completely reduce genetic variability. For example, in Andalucía, there is a high amount of both genetic diversity and clones. This is a result of its chestnut cultivar population being derived from both the northern and central Iberian gene pools. The southern Iberian Peninsula, on the other hand, has the highest clonality, which can be explained by the fact that, in that area, orchards have been modernized to introduce cultivars that are more adapted to the local climatic conditions. Genetic diversity was still conserved because the cultivars came from different origins of genotypes. In Andalucía, clonality resulted in better genotypes for fruit production and adaptation to the drier climate in southern Spain (Pereira-Lorenzo et al., 2011).

Although used much less frequently than grafting, in vitro culture (micropropagation) is another clonal propagating technique used to obtain self-rooted material (Bounous, 2005). When explants are obtained from adult

trees, *Castanea* is one of the most difficult species to establish *in vitro*. However, micropropagation and acclimatization of plantlets obtained from Japanese chestnut seedlings, *Castanea sativa*, and Euro-Japanese hybrids have been successful (Tetsumura and Yamashita, 2004; Chevre and Salesses, 1987). Agri Obtentions in Dijon, France is a largely self-supported INRA (Institut National de la Recherche Agronomique) company that multiplies shoots for chestnut micropropagation. These shoots are sold individually priced to cooperating nurseries, which micropropagate the chestnuts on a commercial scale by using INRA-patented micropropagation techniques (McGranahan, 1992).

Adult chestnut is difficult to propagate by cuttings. Therefore, alternative methods of vegetative propagation of adult chestnut are desirable for tree improvement. Viéitez et al. (1983) have worked with juvenile material to discover under what conditions buds of shoot tips of adult chestnut might give rise to multiple shoots capable of rooting *in vitro*. They successfully established *in vitro* cultures of adult chestnut using stump sprouts as the starting material. Stump sprouts were chosen for *in vitro* culture because, although the material is obtained from adult trees, it is juvenile material. For their plantlet regeneration trials, they found that chestnut requires the ammonium ion for optimal growth *in vitro*. They also found that the treatments that produced the best results in juvenile material also gave good results with cultures initiated using adult chestnut material (Viéitez et al., 1983).

Somatic embryogenesis is a desirable alternative propagation strategy to conventional vegetative propagation of chestnut, which has numerous difficulties associated with it. However, somatic embryogenesis has usually produced low rates of conversion into plantlets. One reason that this method would be sought after is that it could be used to transfer anti-fungal genes into chestnut cells, which could then be used to generate transgenic plants. This process would be much faster than traditional plant breeding efforts to produce blight and/or ink-disease-resistant trees (Coredoira et al., 2008).

Somatic embryo/seedling (SE/SS) technology has been touted as the path that has the highest potential for *in vitro* mass propagation of elite cultivar genotypes (Maynard et al., 2008). As somatic embryos contain in large part dividing cells and therefore have the potential to generate complete plants, their use for genetic transformation has produced important discoveries in the genetic engineering of trees. An efficient method to proliferate *C. sativa* has been developed that cultures *Agrobacterium tumefaciens* carrying marker genes with chestnut somatic embryos. However, such genetically transformed embryos, in general, have a lower conversion rate into whole plants than plantlets obtained in a genetically untransformed line (Maynard et al., 2008).

Embryonic cultures have been identified as the ideal target tissue for transformation (Maynard et al., 2008). At the State University of New York College of Environmental Science and Forestry (SUNY-ESF) in Syracuse, NY, a novel method of inoculating chestnut somatic embryos using *Agrobacterium*-mediated transformation was developed. This method pours the *Agrobacterium* inoculum onto the embryos while they are on multiplication medium. This design was shown to improve *Agrobacterium*-mediated transformation frequency. Rothrock et al. (2007) also demonstrated that the routine practice of wounding tissues prior to co-cultivation was counterproductive or at least unnecessary for transforming chestnut somatic embryos (Rothrock et al., 2007).

Somatic embryogenesis has been almost predominantly induced from zygotic embryos. Merkle et al. (1991) reported somatic embryogenesis in tissue cultures initiated from whole ovules of *C. dentata*. Their results demonstrated that American chestnut can be regenerated via somatic embryogenesis (Merkle et al., 1991). However, the advantages of inducing somatic embryogenesis from leaf tissue, as opposed to zygotic embryos, are that selected mature genotypes could be a source of explants, no sterilization procedure is required, and experiments can be conducted year round. Unfortunately, somatic embryos have poor conversion rates into plantlets. Pre-germination treatments are often required such as cold storage, application of plant hormones, and partial desiccation (Corredoira et al., 2006). Chestnut embryos need cold stratification to germinate. Thus, a period of cold storage enhances chestnut somatic embryos conversion into emblings (plants derived from somatic embryos) (Viéitez, 1999).

The more common method for large-scale vegetative propagation of chestnut, especially of *Phytophthora*-resistant chestnut, is stooling (Viéitez, 1992). Fabbri et al. (1992) reported that girdling the lower portion of the shoots and etiolating produced a greater reaction of adventitious rooting in chestnut propagation by stoolbed technique as opposed to just girdling and etiolation treatments alone (Fabbri et al., 1992). Etiolation, which is the exclusion of light, enhances rooting of chestnut cuttings. Success is substantially accelerated by the addition of auxins, a family of plant hormones. The formation of new roots on chestnut shoots is also favored by the abundance of parenchyma and storage tissues, and less sclerenchyma (Biricolti et al., 1992), probably because sclerenchyma acts as a mechanical barrier for emerging adventitious roots (Viéitez, 1992).

The inability of mature chestnut cuttings to root may be due to root inhibitors. Juvenile cuttings, on the other hand, are easily rooted; and, they not only have very little root inhibitors, they have root promoters. Root inhibitors start developing at about 5-8 months (Viéitez, 1992). In a rooting experiment of Euro-Japanese hybrid chestnut cultivars by Craddock et al. (1992), a fog propagation system was tested against different levels of indole-

3-butryic acid (IBA) applied to chestnut cuttings. The treatment that resulted in the best rooting of chestnut cuttings took place in May with 4000 ppm IBA applied. More importantly, the study's findings emphasized that exogenous auxins were essential to rooting and that fungicide application contributed to rooting of chestnut (Craddock et al., 1992).

Tetsumura et al. (2008) investigated conditions which may be optimal for the rooting of softwood cuttings and found that all clones tested showed higher rooting percentages in 5- and 10-cm long cuttings than in 20-cm long cuttings. A high-humidity fog system resulted in a higher frequency rooting than a mist system. The cuttings rooted better when taken in late June and July than in August. Cuttings planted in Metro-Mix® had higher rooting percentages than in Bora-tsuchi (volcanic tuff). These results provide some insight into possible methods to mass propagate elite rootstocks and own-rooted trees (Tetsumura et al., 2008).

Cultivars of *Castanea mollissima*

The smallest tree of all the commercial species, *C. mollissima* grows to about 12m tall. The nuts are of good quality and tend to be medium-sized. The grafted *C. mollissima* tree matures quickly, generally producing nuts in about three years (Fulbright, 2012). In the United States, selection of *C. mollissima* cultivars as a food crop began in 1930 via cooperators who had obtained trees from the USDA. Cultivars are self-sterile, so pollinizers of different seedlings or cultivars are necessary for fruit production (Payne et al., 1982).

The attractiveness of *C. mollissima* as a nut crop lies in its general precocity, blight resistance, and cold tolerance. There is room to expand this niche market as the majority of growers (64%) in the US have orchards less than approximately ten acres. However, the limitations to growing this crop commercially are significant and they include labor costs, small yields (especially in the first few years of orchard establishment), cost-prohibitive harvest equipment, and important pests and diseases. Fortunately, the results obtained from ongoing research are improving the economic practicality of this nut crop. Warmund (2011) suggested that grafting cultivars onto dwarfing rootstocks, boosting nutritional uptake, and thinning secondary (2º) flowers will improve production (Warmund, 2011).

Systematic evaluations of Chinese cultivars are stymied by the fact that the USDA national germplasm system does not have a large collection of Chinese chestnut. Fortunately, HARC (Horticulture and Agriculture Research Center in New Franklin, Missouri) has 65 named Chinese chestnut cultivars in their repository. They

recommend ‘Sleeping Giant’, ‘AU-Homestead’, ‘Gideon’, ‘Eaton’, and ‘Peach’ cultivars which generally produce 59 to 34 nuts/kg. It is important to correctly identify cultivars genetically to avoid misnaming, homonyms, and synonyms. Collaborations between the University of Missouri, Notre Dame and HARC have discovered several possible mistakes in labeling at HARC. However, they have made cultivar genotype confirmation more accurate through DNA fingerprinting and microsatellite analysis (Warmund, 2011).

Cultivars of *Castanea dentata*

Native to the Appalachian forests, the American chestnut’s (*C. dentata*) former range extended from Maine to Georgia and as far west as Louisiana and Michigan. It is a timber-type species, formerly attaining heights of more than 30m and trunk diameters of 90 to 150 cm. Although it has small nuts (about 77 nuts/kg), some report them to have the sweetest flavor (Russell, 1987). Considering that its former range included Hamilton County, Tennessee (Hardin et al., 2001), it is probable that the area is suitable for growing chestnuts commercially. Indeed, there is some overlap in research directed toward restoration of the American chestnut and chestnut agricultural research.

There are no samples left of cultivars which had American genes in their ancestry. One exception, however, is ‘Paragon’. The American x European variety ‘Paragon’ was highly prized as an orchard tree in the late 1890’s and early 1900’s because it yielded good nuts and grew vigorously. Before the blight came to Lancaster County, Pennsylvania, nurseryman Mr. John G. Reist grafted 800 acres to ‘Paragon’ (Smith, 1950). C.K. Sober developed ‘Paragon’ from a chestnut tree that bore high-quality, large nuts on his farm in Northumberland Co. PA. (Sober, 2009).

The Sober ‘Paragon’ chestnut was trade-marked in 1908 under the U.S. Commissioner of Patents. Colonel Sober’s farm was remarkable for transforming great areas of un-tillable mountain land into valuable crop-producing land using chestnut trees. Sober’s ‘Paragon’ chestnut was marketed as being “Sweet as the native nut (American (*C. dentata*)) and much larger (Reed, 1915).” ‘Paragon’ ripens at around the 23rd of September and has large (≈16g) nuts (Anagnostakis, 2012). Fortunately, it may still be possible to salvage the superior genes of ‘Paragon’ by using it as a mother tree to make crosses with more blight-resistant father trees. Mike Nave, a chestnut researcher, is fairly certain that he re-discovered Paragon in California, although presently there is no way to do a comparative genetic analysis (Nave, 2012).

In general, *C. dentata* as a commercial species was quite different from intensively managed orchards in Europe and Asia. In Appalachia, chestnuts were primarily harvested from wild populations; and, according to several interviews and memoirs, American chestnut orchards in Appalachia were often natural stands that had been cleared of competition and underbrush. This practice provided more room in the canopy and allowed the trees' crowns to spread. Farm animals foraging in these groves helped maintain and manage the undergrowth. Thus, a wild stand became agricultural. These "orchards", although frequently treated as commons even on private property, often had access to them restricted by landowners (Lutts, 2007). Susan Freinkel explains the story of the American chestnut thoroughly in her book *American Chestnut: The Life, Death, and Rebirth of a Perfect Tree* (Freinkel, 2007).

Subsequent to the decline of the canopy-dominant *C. dentata*, a variety of species replaced its former ecological role, including *Quercus prinus*, *Oxydendrum arboretum*, *Acer rubrum*, *Tsuga canadensis*, *Cornus florida*, and *Liriodendron tulipifera*. *Castanea dentata* has persisted as an understory species in some forest ecosystems through repeated cycles of infection, die-back, and re-sprouting (Elliot and Swank, 2008). Also, *C. dentata* can survive for extended periods of time in the subcanopy, that is, without sprouting from the base from former canopy trees or vegetative reproduction. Indeed, many living American chestnut sprouts surveyed after chestnut blight was discovered sprouted before chestnut blight had been introduced in the area surveyed. Thus, *C. dentata* can exist in the understory for decades as a constrained seedling without being associated with the stumps of former canopy trees until they are released from competition by a clear-cut or other break in the canopy (Paillet, 1993).

Nonetheless, some reduction in the density of *C. dentata* may indicate that the sprouting ability of *C. dentata* has declined. Reliable historical information about the distribution and abundance of *C. dentata* pre-blight is unfortunately quite rare. Most studies on forest structure occurred by taking censuses of existing chestnut stumps (Elliot and Swank, 2008). Without human intervention, living *C. dentata* biomass would likely continue to decrease as sexual reproduction has been almost completely eliminated and existing root sprout clones would continue to be progressively eliminated by disease and competition (Paillet, 1993).

Japanese, European, and European-Japanese interspecific hybrids

Among the chestnuts grown in the US are *C. mollissima* cultivars and European-Japanese interspecific hybrids and cultivars. However the range of European cultivars mostly extends to the western portion of the US (Warmund, 2011). As for *C. crenata*, the Japanese species, it is considered an important source of disease resistance

(Burnham, 1988). The European chestnut tree, on the other hand, has characteristics different from that of *C. crenata*. A native of the temperate mountains of west Asia, North Africa, and Europe, *C. sativa* seems to be rather tolerant of a wide range of conditions that are less than optimal. However, *C. sativa* does not grow well on limestone soils (Conedera et al., 2004). *Castanea sativa*, or the sweet chestnut, is most prevalent in the Mediterranean region where it is autochthonous to the area (Mujić et al., 2010). Most of the nuts imported to the U.S. are European, as the nuts are large and of excellent flavor. They are also large trees, reaching heights of 18-24m, and have compact crowns (Vossen, 2000). Due to the active human management of the sweet chestnut in Europe, it has been established at the ecological boundaries of its potential range.

The introduction of Euro-Japanese hybrids into Cuneo, an Italian region that heavily depended on chestnuts, was intended to meet a variety of objectives: to confer genetic resistance to chestnut blight and ink disease, shorten the vegetative phase of new orchards with precocious (early-maturing) cultivars, increase production efficiency by using dwarfing plants and higher density plots, prolong the harvest period by growing early-ripening cultivars, and administer well-described and improved germplasm to local growers and nurseries (Craddock and Pellegrino, 1992).

In an evaluation by Craddock and Pellegrino (1992) of Euro-Japanese hybrids in Cuneo, 40 clones were evaluated for leafing out, blooming, harvesting, peel-ability, and cumulative production totals. Early-ripening hybrids including ‘Bouche de Betizac’, ‘Marsol’, and ‘Précoce Migoule’ were recommended for their resistances to *C. parasitica* and *Phytopthora* spp. Additional cultivars were added in subsequent years (Craddock and Pellegrino, 1992).

The earliest cultivar to leaf out in the spring was ‘Maraval’, which makes it highly susceptible to late spring frosts. ‘Primato’ was the earliest-blooming cultivar. Several European cultivars are male-sterile; for example, all of the Italian ‘Marrone’ cultivars are astaminate. Most of the cultivars are protandrous (male pollen was shed before female flowers were receptive) except for ‘Bournette’ and ‘Marlhac’ which had pistils in bloom before pollen was shed. ‘Primato’ and ‘Tanzawa’ were the earliest ripening cultivars, ripening on the first to the fifteenth of September. The late-ripening hybrids, ‘Vignols’, ‘Maraval’ and ‘Marlhac’, ripen during the first two weeks of October. This is the same period that local European cultivars ripen, which would increase the supply of chestnuts in the market at the same time and would be a disadvantage for the grower/seller (Craddock and Pellegrino, 1992).

Not all of the Euro-Japanese hybrids showed resistance to chestnut blight. The most blight-tolerant cultivar was ‘Marsol’. Fortunately, even if a cultivar is susceptible, application of hypovirulent strain to the fungus seems to render sufficient protection against blight. Overall, the hybrid cultivars ‘Marsol’, ‘Précoce Migoule’ and ‘Bouche de Bétizac’ were recommended for their nut size, and resistance to chestnut blight and ink disease. ‘Garrone Rosso’ (a *C. sativa* cultivar local to Cuneo), ‘Marigoule’, and ‘Marsol’ were also proposed for future plantings for their dual-purpose uses (Craddock and Pellegrino, 1992).

CHAPTER III

INTRODUCTORY CONSIDERATIONS FOR COMMERCIAL CHESTNUT PRODUCTION IN TENNESSEE

Chestnut orchards have been grown in the southeastern United States since the late 1920s. Growers of these initial commercial orchards found *C. mollissima* to be well adapted to the southeastern United States' ecological conditions. Most of these early plantings consisted of *C. mollissima* seedlings, but some of them contained grafted cultivars, such as 'Nanking', 'Meiling', and 'Kuling'. Crops from the southeastern United States' chestnut orchards were sold in large metropoli such as Atlanta and could reach the markets in September, which is much sooner than imports. However, the warm, humid climate of the Southeast made post-harvest spoilage a significant problem, even when nuts were harvested frequently. Other issues included inadequate advertising and inconsistent supplies. Many of these challenges still impede chestnut industry success in the United States today. What may be advantageous for a southern chestnut industry, however, is that southern orchards tend to produce larger nuts (Wallace, 1992).

Chestnut research has been conducted in Tennessee for over a hundred years (Ashe, 1912). Although much of this research has been directed toward developing a blight-resistant Chinese-American chestnut hybrid with the American chestnut timber-type form, this research required planting *C. mollissima* as part of the breeding process (Schlarbaum et al., 1992), and most commercial chestnut orchards in the United States are based on *C. mollissima* (Miller, 2003). Other chestnut research objectives in Tennessee include evaluating existing cultivars for their pest and insect resistance and developing protocols for mass clonal propagation. Various federal and state agencies have been involved in this research. In Tennessee, Tennessee Valley Authority (TVA) established plantations of *C. mollissima* seedlings and hybrids in the 1940s (Schlarbaum et al., 1992).

We are still mostly uncertain as to how commercially available chestnut cultivars and varieties will perform in Tennessee. Approaching this problem may require comparative orchards in different locations (Schlarbaum et al., 1992). Although we anticipate *C. mollissima* cultivars to be the most likely candidates for commercial chestnut production in Tennessee, it may be worth noting that there are two main chestnut growing regions in China from which many *C. mollissima* cultivars come, the northern and southern regions (Burnett, 1987). The southern region,

located along the Yangtze River, is known for its large chestnuts (14 g/nut or greater). Cultivars developed from the southern region may be optimal for growing in Tennessee, as the region is approximately within the same latitude as Tennessee. Korean cultivars also may perform well in Tennessee, as most of Korea's chestnut orchards are south of 36 degrees latitude, which is within Tennessee's latitudinal range of 34-36° latitude (Burnett, 1987).

There are few commercial chestnut growers in Tennessee. The two to four orchards that exist are small-scale and are managed by part-time hobbyists (Coleman, 2013; Welborn, 2013). Tennessee commercial chestnut growers are for now too small-scale to invest in harvesting, processing, or technologically sophisticated storage equipment. There are not many existing marketing outlets for wholesale chestnuts, but some grocers and upscale restaurants sell imported chestnuts or have chestnuts as an ingredient on their menus. Farmers' markets are very promising as initial chestnut marketing outlets as customers have more personal interactions with growers and are able to ask questions on how to properly prepare and store chestnuts. Fine dining establishments also are businesses that can introduce a greater number of consumers to chestnuts (Welborn, 2013).

CHAPTER IV

MATERIALS AND METHODS

Twenty cultivars each represented by 20 individuals were planted in a randomized complete block design in four blocks in five-tree plots. Each block consisted of 100 trees. The total number of trees in the blocks was 400. Chestnut trees that were commercially available were planted in a 5m in-row x 10m between-row spacing. Our orchard was grown under non-irrigated and mostly non-fertilized conditions. The trees were a mixture of container-grown and bare-root trees; and some of them were grafted *in situ*. The cultivars chosen were of two types: probable cultivars, which are used frequently in the chestnut-growing industry and possible cultivars that might prove productive (based on suggestions from nurserymen and chestnut growers). Between each block and around the perimeter of the plot guard rows were planted consisting of a random assortment of chestnut trees, including *C. pumila* and own-rooted *C. crenata*. These trees, which acted as a germplasm collection, were in guard rows and also were evaluated, but were not part of the original main experiment. The guard rows served as positional controls. Our experimental layout is shown in Figure 1. The experimental orchard is in Ooltewah, Tennessee, at the Hugh Smith Dairy Farm in Hamilton County at 35°13' 45" N 85° 00' 03.97 W, elevation 230 meters.

Tree positions											
Rows	8	9-13	14-18	19-23	24-28	29	30-34	35-39	40-44	45-49	50
A	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard
B	guard	Gideon	Lindstrom-99	Payne	Revival	guard	S. Giant‡	Eaton	Lindstrom-99	Paragon	guard
C	guard	Paragon	S. Giant‡	Nanking	Eaton	guard	Gideon	Mossbarger	Betizac*	Willamette	guard
D	guard	Colossal	Smith	Byron	Amy	guard	Peach	Meiling	*C. henryi*	Shing	guard
E	guard	Meiling	Qing	Peach	*C. henryi*	guard	Qing	Nanking	Byron	Smith	guard
F	guard	Betizac*	Willamette	Mossbarger	Shing	guard	Payne	Revival	Colossal	Amy	guard
G	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard
H	guard	Mossbarger	Qing	Peach	Betizac*	guard	Amy	S. Giant‡	Peach	Meiling	guard
I	guard	*C. henryi*	Payne	Shing	Colossal	guard	Qing	Revival	Gideon	Smith	guard
J	guard	Gideon	Revival	Nanking	Eaton	guard	Mossbarger	Byron	Willamette	Eaton	guard
K	guard	Paragon	Byron	Willamette	Amy	guard	Paragon	Lindstrom-99	Payne	Colossal	guard
L	guard	S. Giant‡	Smith	Meiling	Lindstrom-99	guard	Nanking	Betizac*	*C. henryi*	Shing	guard
M	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard	guard
Rows	8	9-13	14-18	19-23	24-28	29	30-34	35-39	40-44	45-49	50

Figure 1 Cultivar trial layout in 2004 at Ooltewah, Tennessee
*Bouche de Betizac; ǂ Sleeping Giant

The orchard was planted in 2003 by a previous researcher (Alexander, 2005; Craddock et al., 2005). The entire orchard, which includes all the trees in all the rows, dead and missing trees, and room to turn a tractor around at the end of every row, is approximately 3.8 ha in an agriculturally zoned area formerly used for growing corn. Each row consists of 50 positions and the rows are named A-V. The main cultivars in the blocks are shown in Table 2.

TABLE 2 Original chestnut cultivars chosen for the randomized complete block design

Cultivars	**Cultivars**
Amy	Nanking
Bouche de Betizac	Peach
Payne	Qing
Colossal	Revival
Eaton	Shing
Gideon	Sleeping Giant
Byron	Smith
Lindstrom-99	Willamette
Meiling	*C. henryi*
Mossbarger	Paragon

Originally, the experiment was designed as a complete randomized block experiment. However, a series of unanticipated events deleted several of the original cultivars, including Coleoptera: Curculionidae (ambrosia beetle) infestation and more importantly, a late spring freeze in 2007. We therefore analyzed the cultivars as a completely randomized design instead of a randomized complete block design. In 2009 we identified the grafts that had survived from the grafts that had been killed down to the rootstock. This included pruning the rootstock sprouts and locating a graft union. If we were able to determine that the original graft had survived, we marked it by painting it with a white latex paint from the graft union up to the first major scaffold branches. If only the rootstock survived, this was marked with orange flagging. The list of cultivars planted in 2003 is shown in Tables 2 and 3. The trunks of the trees were painted from the ground to the first branches. Cultivars that were "own-rooted" (i.e. grown from rooted cuttings) were painted. This was a useful and quick way to distinguish between cultivars, which are clones, and rootstocks, which are derived from seeds.

TABLE 3 Annotated list of chestnut accessions represented or lost in the Smith Farm orchard as of Summer 2012

Cultivar	Quantity	Comments
Alachua*	0	A Dunstan hybrid.[1] Our one accession died.
Amy	3	Early ripening. ≈10-15 g/nut.[2] Recommended for peeled, processed nuts.[3] A Greg Miller selection.
Arima x OP	1	Open-pollinated seedling of 'Arima', a *C. crenata* cultivar from Korea which is often used as a standard for comparison of other cultivars as it has good yields, is vigorous, and bears large fruits.[2]
Armstrong	3	Named after the plant breeder of the University of Kentucky in 1980. It has a sweet flavor and upright growth habit. ≈ 80-84 nuts/kg.[1]
AU-Homestead	2	Had the longest ripening period of the University of Auburn selections. ≈ 86 nuts/kg.[1] AU selections were planted in 1935 and released in 1980.[3]
B3F3	1	TACF third backcross from the American chestnut restoration project.
Bisalta	2	Euro-Japanese hybrid imported from Europe by Michael Dolan of Burnt Ridge Nursery in Washington. ≈ 33-44 nuts/kg.[1]
Blandy x OP	1	Open-pollinated seedling from University of Virginia's Blandy Research Farms.[4]
Bouche de Betizac*	0	A seedling of the French cultivar 'Bouche Rouge' that was pollinated by *C. crenata*. It is pollen sterile, peels well, and is very flavorful. ≈ 33 nuts/kg.[1] None of the 23 grafts survived the first winter (preceding the 2007 freeze). It is reportedly resistant to gall wasp.[27]
Byron	16	Formerly 'Lindstrom-67'. A selection of Jerry Payne from Byron, GA.[5]
C. crenata	3	Japanese chestnut.[6]
C. dentata	2	American chestnut. Susceptible to *C. parasitica*.[7]
C. henryi	5	Henry's chinkapin. Indigenous to China.[8]
C. henryi 'Small'	6	Acquired from Empire Chestnuts. Originally from Nanjing China.[9]
C. henryi 'Yellow'	2	Acquired from Empire Chestnuts. Originally from Nanjing China.[9]
C. mollissima	1	Chinese chestnut.[6]
C. ozarkensis	4	Ozark chinkapin.[6]
C. pumila	17	Chinkapin; bears one nut per bur.[10]
California rootstock	13	Graft failures from Lucienne Grunder. Look markedly different from *C. mollissima* or *C. crenata.* We decided to observe them for a period of time before re-grafting since they appear unique.
Colossal	7	Susceptible to blight. ≈ 30 g/nut.[11]Predominant cultivar grown in California. Euro-Japanese hybrid. Often grown alongside 'Silverleaf'. Pollen sterile.[34]
Colossal x OP	3	Open-pollinated seedling of 'Colossal'.
D2-26-176 x OP	1	B3F3 from the Clapper seed orchard in Meadowview.
D5-26-131 x OP	4	B3F3 from the Clapper seed orchard in Meadowview.
Daebo	2	Korean (*C. crenata/C. mollissima* hybrid) cultivar which is resistant to chestnut blight.[12]
Daebo x OP	6	Open-pollinated seedling of 'Daebo'.
Dunstan seedling	8	A hybrid of *C. mollissima* and *C. dentata*, 'Dunstan' was developed by plant breeder Dr. R.T. Dunstan. It was marketed nationwide as being large-nutted as well as a producer of high-quality timber, and the first chestnut tree to receive a U.S. plant patent.[13]
E-5 x OP	1	Open-pollinated seedling of a Bendabout *C. dentata* which was originally transplanted from Lookout Mountain, GA.

Table 3 continued

Cultivar	Quantity	Comments
Eaton	7	Nuts have a sweet flavor. ≈ 12 g/nut.[11]
Eaton River	6	Ortet was originally from Connecticut.[14] Early ripening; one of the first trees harvested during the harvest season.
Eaton x OP	1	Open-pollinated seedling of 'Eaton'.
Eurobella	1	A *C. crenata* x *C. pumila* hybrid. Easy to peel and a good pollenizer.[15]
Ford's Sweet	1	Although it is a *C. mollissima* cultivar, its sweet kernel reportedly is similar to *C. dentata*.[14]
Ford's Tall	3	Acquired from John Britain. Recommended for peeled, processed nuts.[16] Intended to be a "timber-type" *C. mollissima.* Named after J. Ford Wilkinson.[17] Has been identified as a late secondary flowering cultivar. That is, it produces many secondary burs.[18]
Gideon	19	Uniformly-shaped, attractive nuts. ≈ 12-18 g/nut.[11] Greg Miller selected 'Gideon' from a planting of *C. mollissima* seedlings in Ohio. His 'Gideon' ortet averages 12-18 g/nut. He also selected 'Amy' and 'Peach'.[3] Of all three that we planted, 'Gideon' had the most surviving grafts.
Ginyose	1	A *C. crenata* late-ripening cultivar. May have some resistance to gall wasp[19], but it is susceptible to *Phytophthora katsurae* Ko & Chang.[20]
Heritage	1	Was bred by R.T. Dunstan. It has a timber form and is an American hybrid.[1]
I-8	1	From Bendabout Farm in McDonald, TN. Original ortet has died of *Phytophthora* spp.
Ishizuki x OP	1	Open-pollinated seedling of 'Ishizuki'.
J3 x OP	3	Open-pollinated seedling of Bendabout J3. Bendabout J3 is an open-pollinated Clapper 2nd backcross (B2) from Meadowview.
Jersey Gem	3	A very dwarfing 'Orrin' x 'Nanking' cultivar developed by Henry Hartmann at the Jersey Chestnut Farm in New Jersey. Hartman selected it for its flavor and storability.[1] The only surviving 'Jersey Gem' is growing in a very shady area of the orchard and hasn't grown very much since it was planted in 2003.
Kohr*	0	Has shown signs of graft incompatibility.[28] We had five originally.
Kuling	1	Released by USDA for blight resistance.[35] From Meadowview, VA.
Layeroka*	0	Blight susceptible.[28] We had five originally.
Lewis Acker Chinkapin x Jayne	3	A cross from a chinkapin at Funk's Farm in Ashe County, which is owned by Louis Acker, and 'Jayne'.[33] 'Jayne' is named after Tom Jayne, a plant propagator who worked at the USDA Plant Introduction Station in Glenn Dale, Maryland. He originated the Jayne hybrid by crossing an Allegheny chinkapin with a Chinese chestnut.[21]
Lindstrom-99	1	From the USDA experiment site in Byron, GA. A selection made by Jerry Payne, although never officially given a cultivar name.[5]
Little Giant	3	Connecticut Agricultural Experiment Station identifies as a possible source of dwarfism, which may increase harvest yields and make for a more compact orchard.[18]
LL3*	0	Lula Lake *C. dentata* grafted onto *C. mollissima* rootstocks.[37] We had one originally.
LL9*	0	Lula Lake *C. dentata* grafted onto *C. mollissima* rootstocks.[37] We had one originally.
Mahogany (TM778)*	0	An undescribed *C. mollissima* selection made by Arthur Graves of the Brooklyn Botanic Garden and Connecticut Agricultural Experiment Station.[23] We had 13 originally.
Maraval	6	Euro-Japanese hybrid from France. Nuts store well.[1]
Marco Morris	1	A B2F2 from Meadowview, VA. Unfortunately, the label was lost in planting the tree. This individual is named after the volunteer who planted it.
Marigoule	6	Euro-Japanese hybrid that is resistant to *Phytophthora* spp. ≈ 44-55 nuts/kg.[1]
Marissard	1	A seedling from 'Laguepie' that was open-pollinated by *C. crenata*.[1] Peels well and produced some of the most attractive nuts of the 2010 and 2011 harvest seasons.

Table 3 continued

Cultivar	Quantity	Comments
Marissard x OP	3	Open-pollinated seedling of 'Marrissard'.
Marron du Var*	0	Stores and peels well mechanically. Average susceptibility to kernel rot.[38] We had two originally.
Marrone de Chuisa Pesio x OP	1	Open-pollinated seedling of 'Marrone di Chiusa Pesio'. A good pollenizer for 'Marrone di Chiusa Pesio' is 'Belle Epine', which also produces a high-quality 'Marrone'-like nut.[22]
Marsol*	0	Euro-Japanese hybrid from France. Produces nuts with a very large hylar scar and peels well.[1] We had 7 originally. In 2006, we had 5 'Marsol' grafts. By 2009 we had none.
Miller's B-99	10	An offspring of a Korean chestnut. Greg Miller selected it because it was a fast-growing, straight-bole tree. He suspects that it may have ink-disease resistance as it is growing in a wet area where its neighboring trees died from what appeared to be ink-disease.[9]
Mossbarger	3	Originally from Kentucky. Productive and consistently bears large crops annually. ≈ 12-14 nuts/kg.[14]
Nanking	14	Lighter color nuts than 'Kuling'. ≈ 13-15 g/nut.[11] The most blight-resistant Chinese cultivar in TACF backcross tests.[23]
Nevada*	0	*C. crenata* x *C. sativa*. Commonly used as a source of seedlings according to a nationwide survey of chestnut growers.[39] We only had one originally.
Norris	1	The publisher of *Hort Ideas* magazine, Greg Williams, who also founded the International Tree Crops Association in Norris, TN, noted that 'Norris' seemed to have fewer weevils than other chestnut trees growing in Gravel Switch, KY.[24] Norris, TN was the site of a TVA experiment station. Interestingly, the female flowers have been observed to bloom before the males.[25]
O7 x Daebo	5	Cross between Bendabout Farm's 'O7' and 'Daebo'.
O7 x I-11	4	Both parents are from Bendabout Farm in McDonald, TN. Original ortets have died of *Phytophthora* spp.
Okei*	0	Selected by Kay Ryugo, a UC Davis professor. A *C. crenata* x *C. pumila* hybrid, it is a seedling of 'Silverleaf'. ≈ 26 g/nut.[17] We only had one originally.
Paragon	2	Developed by C.K. Sober. Originally from Northumberland Co., PA. It bore high-quality, large nuts.[26]
Paragon x OP	2	Open-pollinated seedling of 'Paragon'.
Payne	10	Formerly known as Byron 3-3, it was the largest-nutted tree grown in the Beltsville orchard at the USDA's Fruit and Tree Nut Research Station in Byron, GA. It is named after Dr. Jerry Payne who cared for the trees until his retirement in 1994. ≈ 7-8 nuts/kg.[1]
Payne x *C. pumila*	2	A cross between 'Payne' and *C. pumila*.
Payne x OP	1	Open-pollinated seedling of 'Payne'.
Peach	6	A *C. mollissima* selection from Greg Miller at Empire Chestnut. In Michigan, it produces large yields of chestnuts. However, it is very vulnerable to low temperatures.[27] Bears light-colored, fuzzy chestnuts. ≈ 15-22 g/nut.[11] Has demonstrated a decrease in nut size as yields increase.[16]
Precoce Migoule*	0	Euro-Japanese hybrid that produces good nuts but some with multiple embryos. Not a good pollenizer although it does produce pollen. ≈ 57 g/nut.[1] We had three originally.
Primato	1	An early-bearing Euro-Japanese hybrid. ≈ 71-75 nuts/kg and 13-14 g/nut.[1] Purchased as a grafted tree from Burnt Ridge Nursery.

Table 3 continued

Cultivar	Quantity	Comments
Qing	13	Graft failure has been reported; and, it has demonstrated a decrease in nut size as yields increase.[28] Was named by Mike Nave and propagated in 1998. The original ortet is of unknown origin and is growing as a yard tree in Hickory, Kentucky, where it still bears large crops of large-sized(≈20-25g/nut) nuts.[3]
Qing x OP	8	Open-pollinated seedling of 'Qing'.
rootstock	212	A variety of *C. mollissima*, *C. crenata*, and *C. dentata* as well as hybrids.
SA319	7	Grafted from a B2F2 at Meadowview.[29]
SA319 x Ginyose	1	An American/Korean hybrid.
SA319 x Ishizuki	2	An American/Korean hybrid.
SA319 x O7	1	Cross between 'SA319' and Bendabout Farm's 'O7'.
SA319 X OP	1	Open-pollinated seedling of 'SA319'.
SA319 x Tanzawa	1	An American/Korean hybrid.
SA330	3	Grafted from a B2F2 at Meadowview.
SA331*	0	A cross of two F2s.[29] We had four originally.
SA333	10	Grafted from a B2F2 at Meadowview.
SA333 x I-8	2	Cross between 'SA319' and Bendabout Farm's 'I-8'.
SA408	5	Grafted from a B2F2 at Meadowview.
SA408 x DAEBO	3	An American/Korean hybrid.
SA408 x OP	3	Open-pollinated seedling of SA408
SA416	2	A cross of two F2s.[29]
SA553*	0	A 'Nanking' Chinese chestnut.[29] We had two originally.
SA606	3	Grafted from a b2f2 at Meadowview.
Sandae x OP	1	Open-pollinated seedling of 'Sandae', a *C. crenata* cultivar from Korea which yields nuts of ≈ 18.2 grams.[30]
Schrader*	0	A *C. sativa* from Michael Dolan, who acquired it from Harry Lagerstedt from the University of Oregon.[21] We had five originally.
Shing	15	With respect to precocity, yield, and vigor, 'Shing' appears to have distinguished itself among the *C. mollissima* cultivars.[31]
Skioka	1	A Gellatly hybrid, it has produced the seedlings 'Layeroka' and 'Cambell'. Despite the fact that many of the Gellatly hybrids are pollen sterile, it is a good pollenizer. ≈ 80-88 nuts/kg.[1]
Skookum	1	In some locations, 'Skookum' has exhibited vigorous growth. In an architectural chestnut tree analysis, 'Skookum' developed the largest crown of the cultivars observed.[32]
Skookum x OP	1	Open-pollinated seedling of 'Skookum'.
Sleeping Giant	6	*C. mollissima* x (*C. crenata* x *C. dentata*)) hybrid. Potentially a large tree. ≈ 11 g/nut.[11]
Smith	2	Originated on the homestead of George Smith in South Carolina. Also known as 'Old Smith'.[1] Selected by Greg Miller. It probably does best in a northern climate.

Table 3 continued

Cultivar	Quantity	Comments
Sweethart seedling	4	Henry Boyd, grandson of the founder of Boyd Nursery, alleged that 'Sweethart' was as good as the original American chestnut, having the timber form and sweet taste of American chestnut. He was alive when chestnut trees were still present in the Southeast US. The original ortet from Ohio was from Mrs. Hart's backyard, which had been planted by her husband. Unfortunately, the nuts are far too small for commercial production. A larger nut is usually more desireable.[21]
Tiger Paw rootstock*	0	From Michael Dolan. Originally from China from the Fa Hua Ssu Temple near Peking as a USDA plant introduction.[40] We had one originally.
Tskuba*	0	Produced by a breeding program in Japan which was formed in 1952 to develop gall-wasp resistant cultivars. Thus, it is reportedly resistant to gall-wasp.[32] We had one originally.
W.C.*	0	From Clifford England Nursery. Spreading habit.[41] We had one originally.
Willamette	4	An R.D. Wallace-patented hybrid chestnut tree that is blight-resistant and produces sweet and easy-to-peel nuts.[42]
Total	**567**	

[1]Nave, 1998; [2]Kim et al., 2008; [3]Miller, 2003; [4]Strong, 2010; [5]Nave, 2012; [6]Bost, 2011; [7]Smart, 1992; [8]Lang et al., 2007; [9]Miller personal communication, 2012; [10]Payne et al., 1993; [11]Fulbright, 2005; [12]Lee et al., 2006; [13]El-Gholl et al., 1996; [14]Masabni et al., 2007; [15]Grunder, 2007; [16]Hunt, 2005; [17]Anagnostakis, 2012; [18]Warmund, 2011; [19]Park et al., 1981; [20]Oh et al., 2007; [21]Craddock personal communication, 2012; [22]Craddock and Pellegrino, 1992; [23]Hebard, 2005a; [24]Williams, 2012; [25]Jones, 2008; [26]Sober, 2009; [27]Fulbright, 2012; [28]Hunt et al., 2006; [29]Hebard personal communication, 2012; [30]Kim, 2006; [31]Craddock et al., 2005; [32]Solar and Štampar, 2005; [33]Gillis, 2010; [34]Vossen, 2000; [35]Smith, 1950; [36]Inoue et al., 2009; [37]Alexander, 2005; [38]Boutard, 2002; [39]Gold et al., 2005; [40]Ryerson, 1928; [41]England, 2005; [42]Wallace, 1990

*Present in the original 2003-2004 planting but subsequently died.

Some of the cultivars for which grafts had failed, but whose rootstocks survived, were re-grafted. They were re-grafted either back to the original cultivar, or to a different cultivar if the original cultivar had an extremely poor survival rate. As of 2012, we had a total of 563 trees, 212 of which were rootstocks, which were composed of a variety of *Castanea* species and hybrids. The germplasm collection comprised those cultivars and species accessions that were not part of the main blocks (see Table 2). We started the main blocks at position nine because the field was not square. The germplasm collection was in rows A, G, M-Q, S, T, U, and V, all positions less than nine in rows A-N and positions 29 and 50 in rows B-F. Some of the trees were completely killed by the 2007 freeze and left an open space. From 2009 to 2011, we planted additional trees in some of these open spaces. In 2010, we re-grafted onto some of the rootstocks by top-working using the whip and tongue grafting method.

Some of the grafts did not survive in the first few years between 2003 and 2006 due to a variety of causes including late spring frosts, ambrosia beetles, and graft incompatibility, but most of the grafts that perished were killed in the freezing temperatures that occurred from April 6-9, 2007, after an unseasonably warm March (Chattanoogan, 2007). The daily minimum air temperature during this period was less than or equal to 2.2°C. The previous month, March, had been unusually warm with an average daily air temperature of 13.8°C, which was 0.9°C higher than the previous record year in 1973. The uncommon temperatures that occurred during this time period, then, did not happen in the April 2007 freeze, as the date of the freeze was not unusually late, but during March, 2007 (Mulholland et al., 2009). The Tennessee Wildlife Resources Agency documented significant damage to populations of white oak and mast crops of other oak species, especially in the lower elevations (Chattanoogan, 2007). The experimental orchard lies on the valley floor at the foot of White Oak Mountain. Although the experiment started out with 20 samples each of 20 populations of cultivars plus the accessions in the guard rows, by 2009 (when I started on the project) the samples' numbers were lowered and were rendered unequal. Because our data were not normally distributed (UNIVARIATE procedure, SAS Inst. Inc.) and we had unequal sample sizes, we used the Kruskal-Wallis non-parametric analysis of variance (chi-square approximation; NPAR procedure, SAS Inst. Inc.) with Tukey-protected alpha levels (α=0.05) and treated the entire experiment as a completely random design consisting of single-tree plots.

The main evaluation of the trees was for their nut quality and productivity, but also harvest data such as dates harvested were collected. The nuts were collected across three harvest seasons: Fall 2009, Fall 2010 and Fall 2011. Harvesting, weighing, and counting were conducted by the author, students, and volunteers. Observations

were made and nuts collected by several field parties systematically surveying the study area on foot and by vehicle. Although nuts were gathered, counted, and weighed in 2008, this was done by a previous researcher (Alexander, 2005; Craddock et al., 2005). Data were not gathered in 2007 as that was the year of the late spring freeze, which was so injurious to the trees that it rendered the majority of them barren of nuts.

The trees were harvested based on visual observation of ripeness or of nuts on the ground. During harvest seasons, nuts were picked from each tree separately. Each tree's production was placed in a paper bag and labeled with the tree's cultivar name, type, or species. Even though some of the grafts had died, nuts from the surviving rootstocks also were harvested to assess whether they might represent a viable new cultivar. In the summer and fall of 2011, we flagged potentially promising rootstocks with white flagging and rootstocks that were producing nuts that were not marketable with blue flagging. The blue-flagged trees were those that we intended to graft later by top-working.

Kruskal-Wallis non-parametric one-way analysis of variance with Tukey-protected alpha levels was performed only on those cultivars for which at least three representatives were sampled in the harvest period from 2008-2011. Cultivars that had less than three representatives sampled in a harvest year are represented graphically in Figures1-9, but were not statistically assessed in the data analysis.

Some cultivars did not have at least three trees harvested in 2008, 2009, 2010, or 2011. In some cases, this may simply have been due to the fact that there were too few trees representing a cultivar because it was a rare accession or some of the accessions had died, so data analysis could not be performed on the cultivar. However, by combining all harvests from 2008-2011, we were able to perform data analysis (Kruskal-Wallis non-parametric one-way analysis of variance with Tukey-protected alpha levels) on most cultivars even if we may not have been able to in any one particular year.

Some of the cultivars for which grafts had failed, but with surviving rootstocks, were re-grafted, either back to the original cultivar, or, if the original cultivar had an extremely poor survival rate, to a different cultivar. Open spaces were being filled in with new cultivars as well. As of 2012, we had a total of 563 trees that consisted of 89 different cultivars.

CHAPTER V

RESULTS

Results are presented for each year 2008-2011 for cultivar nut mass (g/nut) and yield/tree. Significant differences were observed ($p<0.05$) in nut mass and yield between cultivars for all years from 2008 to 2010, for all years 2008-2010 combined, and for nut mass in 2011, but not for yield in 2011 (Table 11). Cultivars are listed in Tables 4-12 in order of rank, starting with the highest ranking cultivar in terms of nut size or yield. The larger-sized nut cultivars in general were 'Colossal', 'Marigoule', 'Shing', 'Nanking', 'Qing', and 'Gideon'. The smaller-sized nut cultivars were 'Eaton River', 'Byron', 'Sleeping Giant', 'Payne', 'Dunstan' seedling, 'SA333', and 'Little Giant'. 'Eaton' produced medium-sized to small nuts.

The cultivars produced different amounts of nuts. The high-yielding cultivars were usually 'SA333', 'Eaton', 'Shing', 'Colossal', 'Payne', 'Marigoule', 'Maraval', 'Gideon', and 'Qing'. The lower-yielding cultivars were 'Nanking', 'Byron', 'Eaton River', 'Little Giant', 'Peach', 'Willamette', and 'Sleeping Giant'. The 2009 harvest year was the best year in terms of both yield and nut size, followed by 2008. The harvests of 2010 and 2011 were the poorer years in terms of both yield and nut size, with 2011 being the worst in both respects.

2008 nut mass and yield

Year 2008 results are based on a harvest of 58 trees, which was only five years after planting. The trees were in their sixth growing season, but had had several setbacks, including the catastrophic freeze of 2007 the year before. In some instances, a cultivar with a lower average of grams per nut may still have a mean rank higher than a cultivar with a greater average of grams per nut. For example, 'Nanking' in 2008 had an average of 18.3 g/nut but scored slightly higher in mean rank, while 'Marigoule' had an average of 19.1 g/nut but scored slightly lower in mean rank. This can be explained by the nature of the Kruskal-Wallis non-parametric test, which uses rank sums instead of means. The rank sums are then arranged in increasing order of magnitude (Zar, 2010). Averages can be affected by extremely low or extremely high values. In 2008, two 'Marigoule' trees produced 13.8 g/nut and 17.4 g/nut, which brought down its averages. Most 'Marigoule' trees in that year were in the 20's in g/nut. The values of

13.8 g/nut and 17.4 g/nut affected the mean rank of 'Marigoule' slightly too. On the other hand, 'Nanking' had produced larger nut sizes of 18.5, 18.8, and 17.8 g/nut. Because all data from all groups are ranked together, this gave 'Nanking' a slightly higher mean rank, although it was not significantly different from 'Marigoule'.

'Colossal' (21.4 g/nut) and 'Nanking' (18.3 g/nut) ranked largest in nut size in 2008. They were significantly larger than 'Gideon' (12.6 g/nut), 'Qing' (12.5 g/nut), 'Sleeping Giant' (11.7 g/nut), and 'Dunstan' seedling (10.6 g/nut), but not than 'Marigoule' (19.1 g/nut), 'Peach' (17.9 g/nut), 'Shing' (17.0 g/nut), 'Payne' (15.5 g/nut), and 'Eaton' (14.6 g/nut). 'Marigoule' (19.1 g/nut) was significantly larger than 'Qing' (12.5 g/nut), 'Sleeping Giant' (11.7 g/nut), and 'Dunstan' seedling (10.6 g/nut), but not 'Colossal' (21.4 g/nut), 'Nanking' (18.3 g/nut), 'Peach' (17.9 g/nut), 'Shing' (17.0 g/nut), 'Payne' (15.51 g/nut), 'Eaton' (14.6 g/nut), and 'Gideon' (12.6 g/nut). 'Peach' (17.9 g/nut) was only significantly larger than 'Dunstan' seedling (10.6 g/nut), but not different from the rest of the cultivars. 'Shing' (17.0 g/nut) was only significantly larger than 'Dunstan' seedling (10.6 g/nut), but not the rest of the cultivars (Table 4).

Table 4 Results of Tukey's Studentized Range (HSD) Test for 2008 cultivar nut mass (grams/nut)

Cultivar	**N Harvested**	**Tukey Grouping***	**Avg. g/nut ± SD**
Colossal	8	a	21.4 ± 5.41
Nanking	3	a	18.3 ± 0.418
Marigoule	5	a, b	19.1 ± 3.20
Peach	3	a, b, c	17.9 ± 2.84
Shing	4	a, b, c, d	17.0 ± 1.07
Payne	7	a, b, c, d, e	15.5 ± 1.74
Eaton	3	a, b, c, d, e	14.6 ± 0.235
Gideon	12	c, b, d, e	12.6 ± 2.17
Qing	4	c, d, e	12.5 ± 0.965
Sleeping Giant	5	d, e	11.7 ± 1.89
Dunstan seedling	4	e	10.6 ± 2.36
Total 11 cultivars	**58**		

*Means with the same letter are not significantly different ($p \geq 0.05$).

Differences among cultivars for yield in 2008 were statistically significant ($p < 0.05$). Kruskal-Wallis non-parametric one-way analysis of variance with Tukey-protected alpha levels showed that mean yields (illustrated in Table 5) were not significantly different from each other except for 'Shing' (1.92 kg/tree) and 'Peach' (0.13 kg/tree). The rank of yield for 'Shing' (1.9 kg/tree) was significantly higher than 'Peach' (0.13 kg/tree), but not significantly different from 'Colossal' (1.3 kg/tree), 'Payne' (0.814 kg/tree), 'Eaton' (0.48 kg/tree), 'Sleeping Giant' (0.82 kg/tree), 'Gideon' (0.44 kg/tree), 'Marigoule' (0.41 kg/tree), 'Dunstan' seedling (0.36 kg/tree), 'Nanking' (0.33

kg/tree), or ‘Qing’ (1.04 kg/tree). ‘Peach’ (0.13 kg/tree), although significantly lower in yield than ‘Shing’ (1.92 kg/tree), was not significantly lower from the rest of the cultivars harvested in 2008. Average nut mass and yield for all cultivars harvested in 2008, including those that may have been represented by one or two trees, are shown in Figures 2 and 3.

Table 5 Results of Tukey’s Studentized Range (HSD) Test for 2008 cultivar yield (kg per tree)

Cultivar	**N Harvested**	**Tukey Grouping***	**Avg. kg/tree ± SD**
Shing	4	a	1.92 ± 0.982
Colossal	8	a, b	1.30 ± 0.826
Payne	7	a, b	0.814 ± 0.392
Eaton	3	a, b	0.48 ± 0.233
Sleeping Giant	5	a, b	0.83 ± 0.806
Gideon	12	a, b	0.45 ± 0.299
Marigoule	5	a, b	0.41 ± 0.248
Dunstan seedling	4	a, b	0.36 ± 0.156
Nanking	3	a, b	0.33 ± 0.129
Qing	4	a, b	1.04 ± 1.58
Peach	3	b	0.13 ± 0.0860
Total 11 cultivars	**58**		

*Means with the same letter are not significantly different ($p \geq 0.05$).

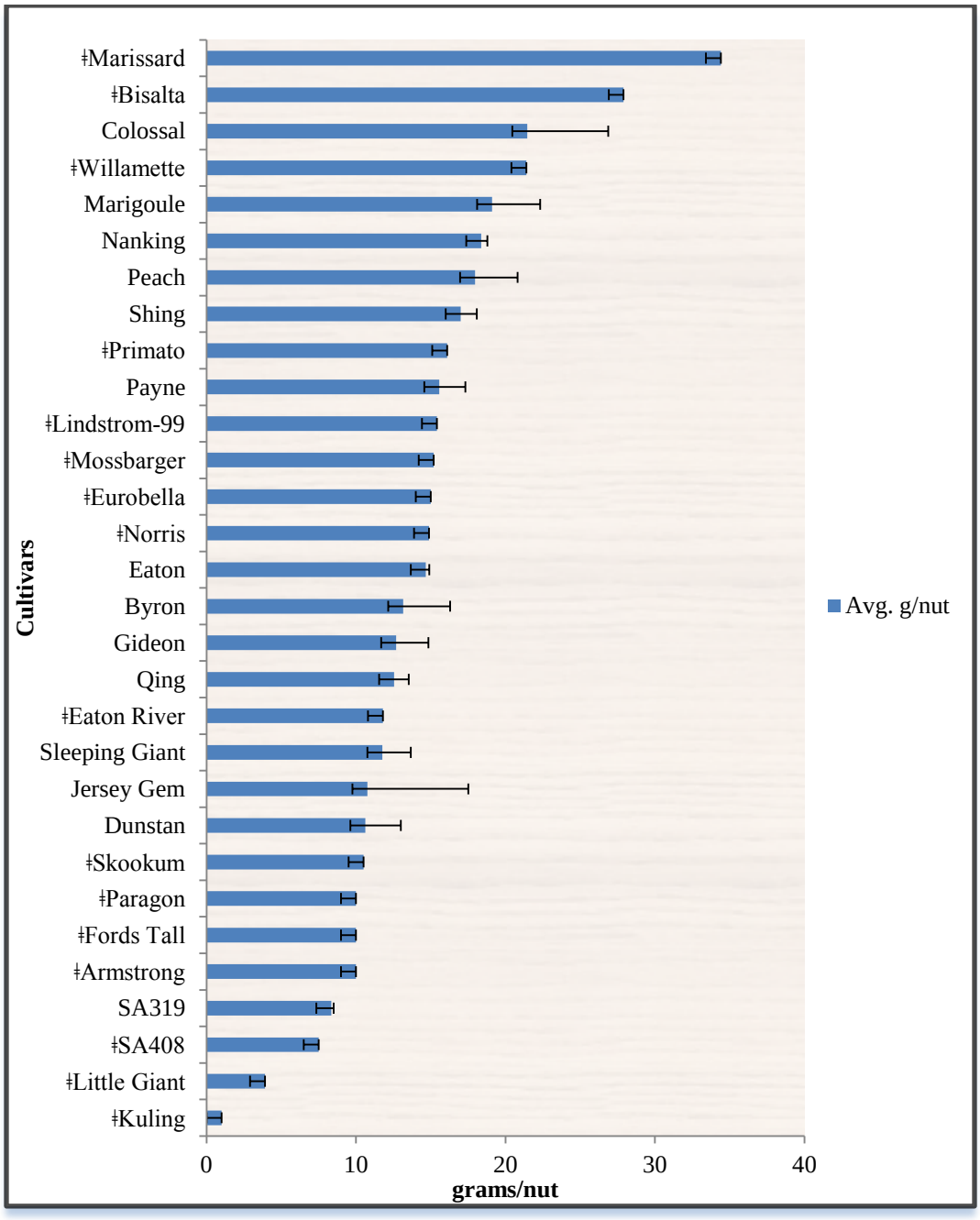

Figure 2 Average grams/nut for 30 chestnut cultivars in 2008; ǂone accession harvested; error bars represent one standard deviation from the mean

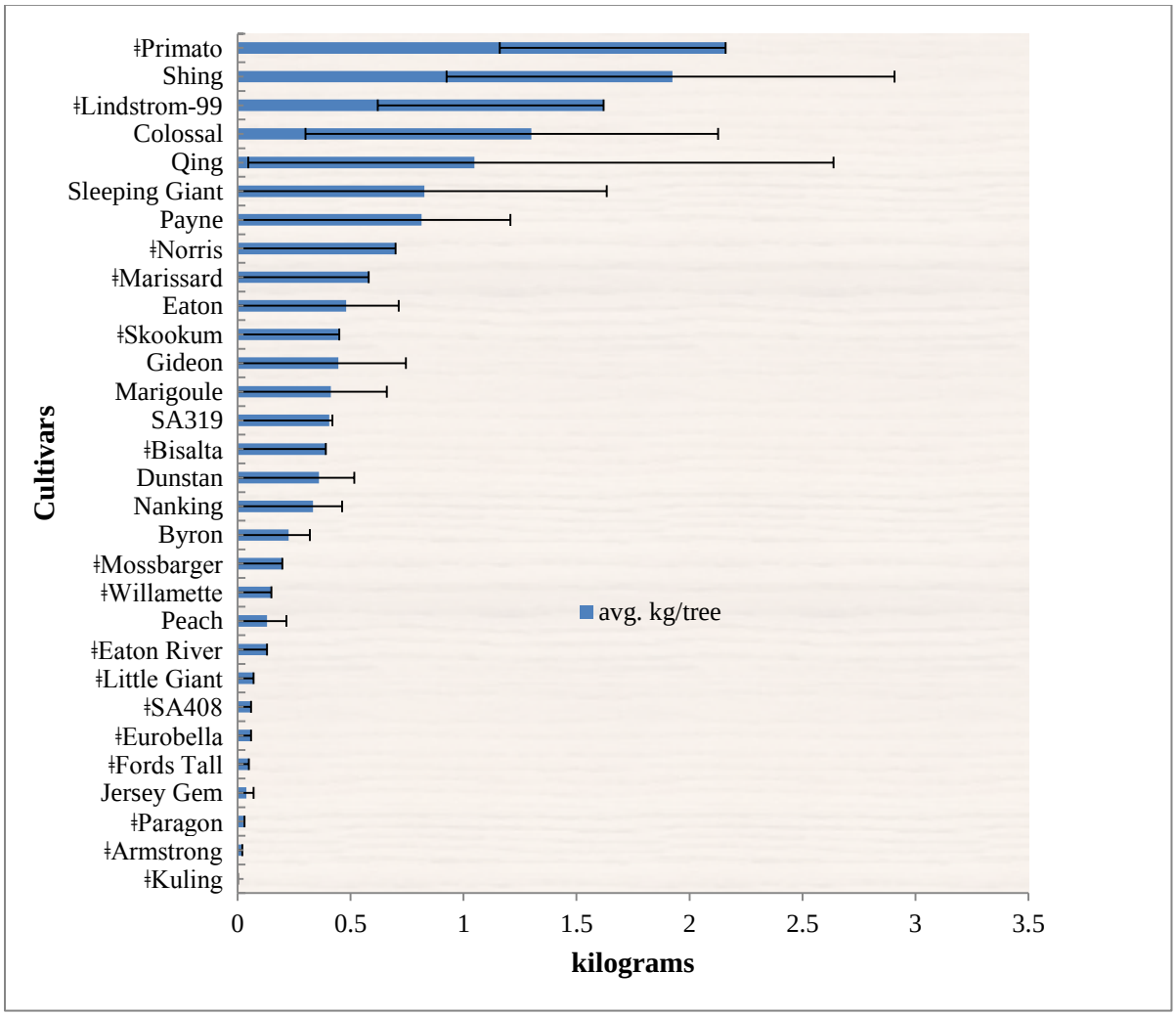

Figure 3 Average yield (kg/tree) for 30 chestnut cultivars in 2008; ǂone accession harvested; error bars represent one standard deviation from the mean

2009 nut mass and yield

Results of the 2009 harvest are presented in Tables 6 and 7, respectively. Data were coupled from 74 trees of 12 cultivars for which three or more trees were harvested. ‘Marigoule’ had the largest nuts (23.6 g/nut), followed closely by ‘Colossal’ (21.8 g/nut), ‘Shing’ (18.3 g/nut), and ‘Qing’ (18.5 g/nut). ‘Marigoule’ (23.6 g/nut) in 2009 had a mean rank of nut size (grams/nut) that was significantly higher than ‘Peach’(15.3 g/nut), ‘Gideon’ (15.3 g/nut), ‘Eaton River’ (14.7 g/nut), ‘Byron’ (13.9 g/nut), ‘Sleeping Giant’ (12.6g/nut), ‘Nanking’ (12.1 g/nut), ‘Payne’ (10.4 g/nut), and ‘Little Giant’ (6.66 g/nut), but not ‘Colossal’ (21.8 g/nut), ‘Shing’ (18.3 g/nut), and ‘Qing’ (18.5 g/nut). ‘Colossal’ (21.8 g/nut) was significantly larger in size than ‘Eaton River’ (14.7 g/nut), ‘Byron’ (13.9 g/nut), ‘Sleeping Giant’ (12.6 g/nut), ‘Nanking’ (12.1 g/nut), ‘Payne’ (10.4 g/nut), and ‘Little Giant’ (6.66 g/nut), but not ‘Marigoule’ (23.6 g/nut), ‘Shing’ (18.3 g/nut), ‘Peach’ (15.3 g/nut), or ‘Gideon’ (15.3 g/nut). ‘Shing’ (18.3 g/nut) and ‘Qing’ (18.5 g/nut) were significantly larger than ‘Nanking’ (12.1 g/nut), ‘Payne’ (10.4 g/nut), and ‘Little Giant’ (6.66 g/nut), but not the rest of the cultivars. ‘Peach’ (15.3 g/nut) was only significantly smaller than ‘Marigoule’ (23.6 g/nut), but not from ‘Colossal’ (21.8 g/nut), ‘Shing’ (18.3 g/nut), ‘Qing’ (18.5 g/nut), ‘Gideon’ (15.3 g/nut), ‘Eaton River’ (14.7 g/nut), ‘Byron’ (13.9 g/nut), ‘Sleeping Giant’ (12.6 g/nut), ‘Nanking’ (12.1 g/nut), or ‘Payne’ (10.4 g/nut). ‘Peach’ (15.3 g/nut) was significantly larger than ‘Little Giant’ (6.66 g/nut) only. ‘Gideon’ (15.3 g/nut) and ‘Peach’ (15.3 g/nut) were equivalent. ‘Eaton River’ (14.7 g/nut) was only significantly smaller than ‘Marigoule’ (23.6 g/nut) and ‘Colossal’ (21.8 g/nut), but not the rest of the cultivars. ‘Byron’ (13.9 g/nut), ‘Sleeping Giant’ (12.6 g/nut), and ‘Eaton River’ (14.7 g/nut) were equivalent in size. ‘Nanking’ (12.1 g/nut) was smaller than ‘Marigoule’ (23.6 g/nut), ‘Colossal’ (21.8 g/nut), ‘Shing’ (18.3 g/nut), and ‘Qing’ (18.5 g/nut), but not the remaining evaluated cultivars in 2009. ‘Payne’ (10.4 g/nut) and ‘Nanking’ (12.1 g/nut) were equivalent in size. ‘Little Giant’ (6.66 g/nut) was smaller than half of evaluated cultivars (‘Marigoule’ (23.6 g/nut), ‘Colossal’ (21.8 g/nut), ‘Shing’ (18.3 g/nut), ‘Qing’ (18.5 g/nut), ‘Peach’ (15.3 g/nut), and ‘Gideon’ (15.3 g/nut)) but statistically equal to the rest of the cultivars analyzed as shown in Table 6.

Table 6 Results of Tukey's Studentized Range (HSD) Test for 2009 cultivar nut mass (grams/nut)

Cultivar	N Harvested	Tukey Grouping*	Avg. g/nut ± SD
Marigoule	5	a,	23.6 ± 2.92
Colossal	8	a, b	21.8 ± 1.90
Shing	8	a, b, c	18.3 ± 5.45
Qing	5	a, b, c	18.5 ± 1.89
Peach	3	b, c, d	15.3 ± 1.31
Gideon	12	b, c, d	15.3 ± 4.20
Eaton River	5	c, d, e	14.7 ± 1.93
Byron	8	c, d, e	13.9 ± 3.10
Sleeping Giant	3	c, d, e	12.6 ± 3.93
Nanking	5	d, e	12.1 ± 2.53
Payne	9	e, d	10.4 ± 2.48
Little Giant	3	e	6.66 ± 1.27
Total 12 cultivars	**74**		

*Means with the same letter are not significantly different ($p \geq 0.05$). Our best year in cultivar nut mass was 2009. Over half of the cultivars averaged 15 g/nut or higher.

There were 11 'SA333' trees harvested in 2009. Due to the fact that 'SA333' and 'SA606' were so fruitful, we were unable to count every nut from each tree. The total harvest from all of the 'SA333' trees in 2009 was 40.54 kg, which averages out to 3.685 kg per tree. Only one 100-nut sample was taken of 'SA606' to get an estimate of the nut size, which was 11.1 grams per nut (90 nuts/kg), which indicates very small nuts not practically marketable for fresh consumption. Similar to the other 'SA' trees, in 2008 'SA408' had an average of 7.5 grams/nut and only one tree of 'SA408' was harvested.

The one Japanese rootstock (*C. crenata*) that was harvested in 2009 (Figure 3) had a higher nut mass (20.4g) than the rootstocks' average (15.0g). Most of the rootstocks are *C. mollissima.* The highest variability in fruit mass was in the rootstocks (COV 45.7%) and the lowest variability was in 'Ford's Tall' (COV 6.78%).

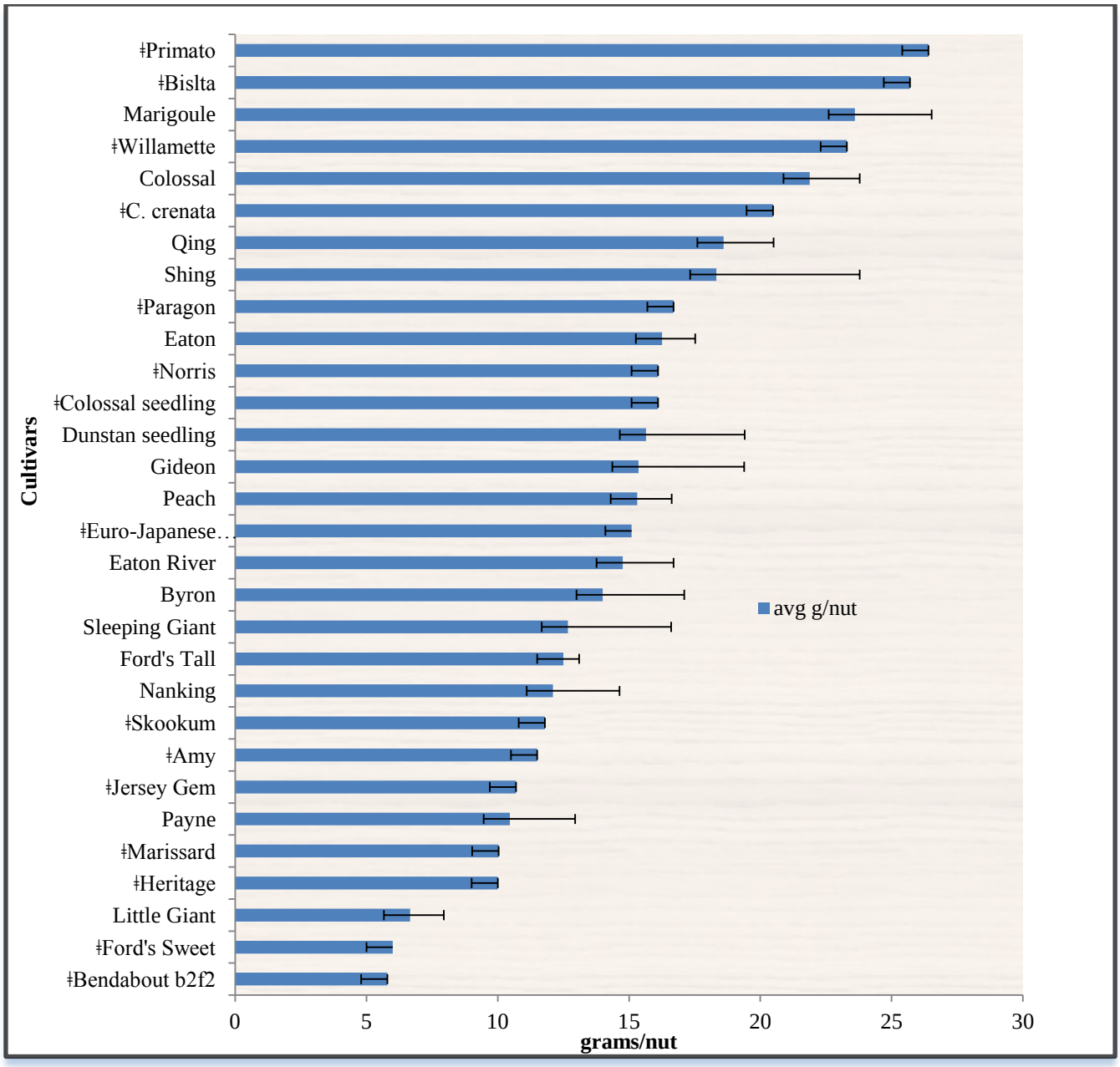

Figure 4 Average grams/nut for 30 chestnut cultivars in 2009 and *seedling rootstocks; ‡one accession harvested; error bars represent one standard deviation from the mean

Even though there was only one 'Primato', it had the largest nuts (26.4g) in 2009. However, it subsequently died in 2012. Figure 3 shows the average grams per nut for harvested cultivars in 2009.

Of the eight 'Colossal' trees harvested in 2009, there was one tree that had an average nut size of 26.3 grams that was an extremely high outlier (unranked data). 'Eaton River', of which five trees were harvested, had two outliers on opposite ends of the size spectrum: 18.0 grams and 11.9 grams (unranked data). 'Nanking' had one high outlier (16.8 grams) out of the five trees harvested (unranked data). 'Shing', which was generally amongst the largest-sized nuts, had one low outlier tree that produced nuts that averaged 5.0 g/nut (unranked data).

Differences among cultivars of which greater than or equal to three trees were harvested (Table 7) were significant for yield in 2009 ($p<0.05$).

Table 7 Results of Tukey's Studentized Range (HSD) Test for 2009 cultivar yield (kg per tree)

Cultivar	**N Harvested**	**Tukey Grouping***	**Avg. kg/tree ± SD**
Colossal	8	a	6.25 ± 2.95
Marigoule	5	a, b	3.61± 2.08
Sleeping Giant	3	a, b, c	0.71 ± 0.380
Gideon	12	a, b, c	1.04 ± 0.752
Peach	3	a, b, c	1.73 ± 2.05
Payne	9	a, b, c	1.08 ± 9.30
Shing	8	a, b, c	0.390 ± 0.743
Qing	5	b, c	0.89 ± 0.936
Nanking	5	b, c	0.69 ± 0.570
Byron	8	c	0.244 ± 0.162
Eaton River	5	c	0.24 ± 0.162
Little Giant	3	c	0.21 ± 0.114
Total 12 cultivars	**74**		

*Means with the same letter are not significantly different ($p\geq0.05$).

'Colossal' (6.25 kg/tree) yielded the greatest number of kilograms per tree in comparison with 'Qing' (0.89 kg/tree), 'Nanking' (0.69 kg/tree), 'Byron' (0.224 kg/tree), 'Eaton River' (0.24 kg/tree), and 'Little Giant' (0.21 kg/tree), but not 'Marigoule' (3.61 kg/tree), 'Sleeping Giant' (0.71 kg/tree), 'Gideon' (1.04 kg/tree), 'Peach' (1.79 kg/tree), 'Payne' (1.08 kg/tree), or 'Shing' (0.74 kg/tree). 'Marigoule' (3.61 kg/tree) significantly differed from 'Byron' (0.224 kg/tree), 'Eaton River' (0.24 kg/tree) and 'Little Giant' (0.212 kg/tree) by having greater yields per tree, but not from the other cultivars; and 'Sleeping Giant' (0.71 kg/tree), 'Gideon' (1.04 kg/tree), 'Peach' (1.79 kg/tree), 'Payne' (1.08 kg/tree), 'Shing' (0.74 kg/tree), 'Qing' (0.89 kg/tree), and 'Nanking' (0.69 kg/tree) were equivalent to 'Marigoule'(3.61 kg/tree) in yield. The yields of 'Byron' (0.244 kg/tree), 'Eaton River' (0.24 kg/tree), and 'Little

Giant' (0.21 kg/tree) were significantly smaller than that of 'Colossal' (6.25 kg/tree) and 'Marigoule' (3.61 kg/tree), but not from the other cultivars.

In 2009, 'Marigoule' had one high outlier tree of the five harvested that yielded 7.42 kg (unranked data). One of the five 'Qing' trees was a high outlier that bore 2.62 kg (unranked data). 'Shing' had a similar high outlier tree that yielded 2.43 kg out of the eight 'Shing' trees harvested (unranked data). Of the eight 'Colossal' trees harvested, one tree that produced 1.63 kg was a low outlier within the cultivar (ranked data).Year 2009 was a productive one for 'Colossal' (6.25 kg/tree), as it had the highest average yield (kg) per tree. It also had the 5th largest average nut (21.8 grams) in the same year (Fig. 3 and 4).

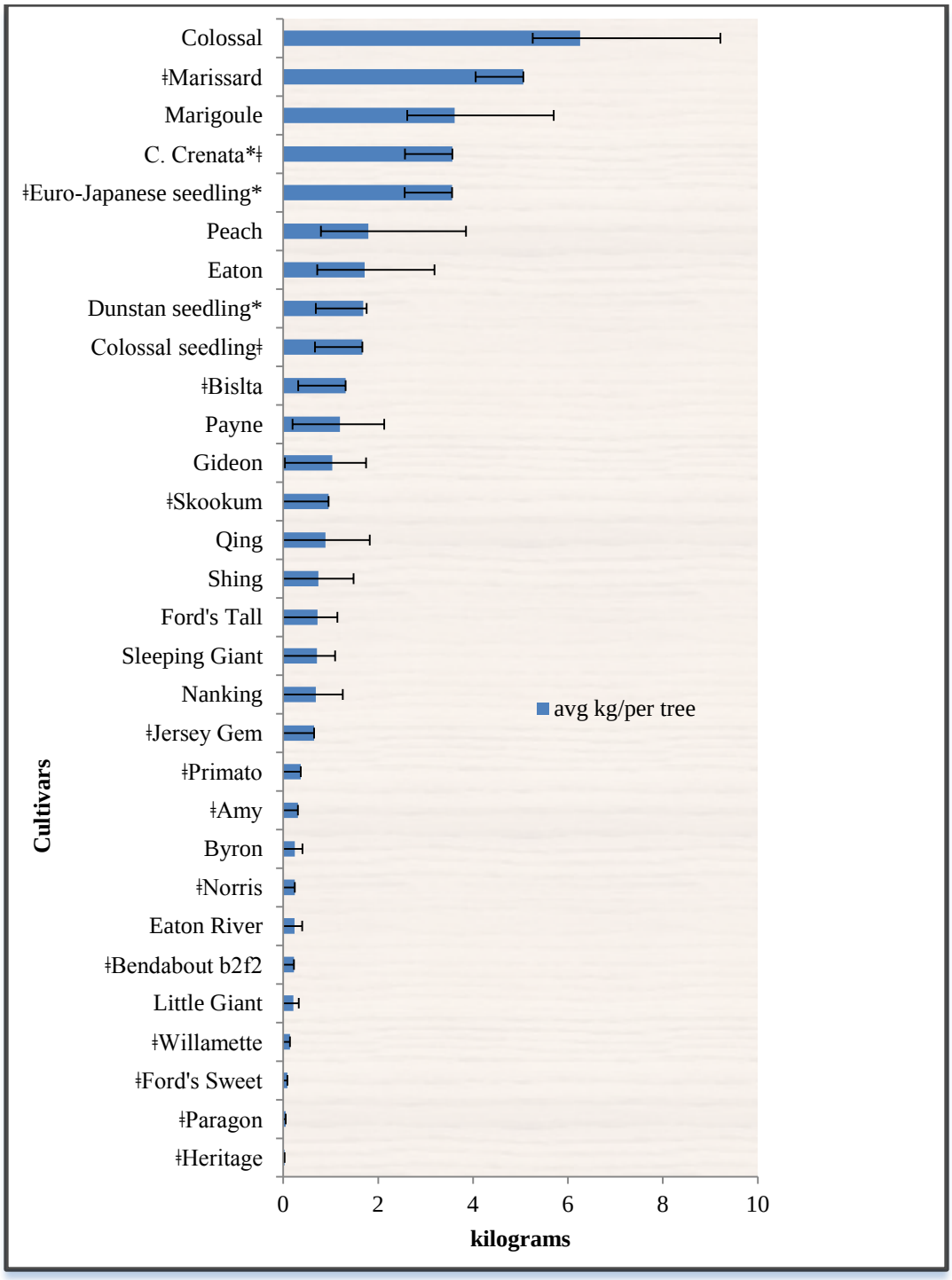

Figure 5 Average yield (kg/tree) for 30 chestnut cultivars and *seedling rootstocks in 2009; ‡one accession harvested; error bars represent one standard deviation from the mean

2010 nut mass and yield

In 2010, a total of 16 cultivars and 99 trees were harvested. The cultivars that were distinguishable in nut size were 'Maraval' (14.9 g/nut), 'Marigoule' (10.8 g/nut), 'Peach' (10.5 g/nut), 'Byron' (9.49 g/nut), and 'Dunstan' seedling (9.25 g/nut). They were all significantly larger than 'Little Giant' (2.71 g/nut), but were indistinguishable from each other. 'Maraval' (14.9 g/nut), was significantly larger than 'Willamette' (6.02 g/nut), 'Shing' (5.30 g/nut), 'SA333' (3.94 g/nut), 'Payne' (4.94 g/nut), and 'Little Giant' (2.71 g/nut), but not 'Marigoule' (10.8 g/nut), 'Peach' (10.5 g/nut), 'Byron' (9.49 g/nut), 'Dunstan' (9.25 g/nut), 'Nanking' (8.64 g/nut), 'Qing' (9.07 g/nut), 'Colossal' (7.82 g/nut), 'Gideon' (7.53 g/nut), 'Sleeping Giant' (7.29 g/nut), or 'Eaton' (6.97 g/nut) as shown in Table 8. All cultivars' nut weights in 2010 are shown in Figure 5.

Table 8 Results of Tukey's Studentized Range (HSD) Test for 2010 cultivar nut mass (grams/nut)

Cultivar	N Harvested	Tukey Grouping*	Avg. g/nut ± SD
Maraval	5	a	14.9 ± 4.71
Marigoule	4	a, b	10.8 ± 1.63
Peach	3	a, b	10.5 ± 1.37
Byron	10	a, b	9.49 ± 2.87
Dunstan seedling	6	a, b, c	9.25 ± 3.61
Nanking	4	a, b, c	8.64 ± 2.22
Qing	6	a, b, c	9.07 ± 3.57
Colossal	9	a, b, c	7.82 ± 3.47
Gideon	13	a, b, c	7.53 ± 1.80
Sleeping Giant	5	a, b, c	7.29 ± 1.16
Eaton	3	a, b, c	6.97 ± 0.805
Willamette	3	b, c	6.02 ± 0.874
Shing	10	b, c	5.30 ± 1.80
SA333	9	b, c	3.94 ± 1.76
Payne	6	b, c	4.94 ± 1.75
Little Giant	3	c	2.71 ± 0.103
Total 16 cultivars	**99**		

*Means with the same letter are not significantly different ($p \geq 0.05$).

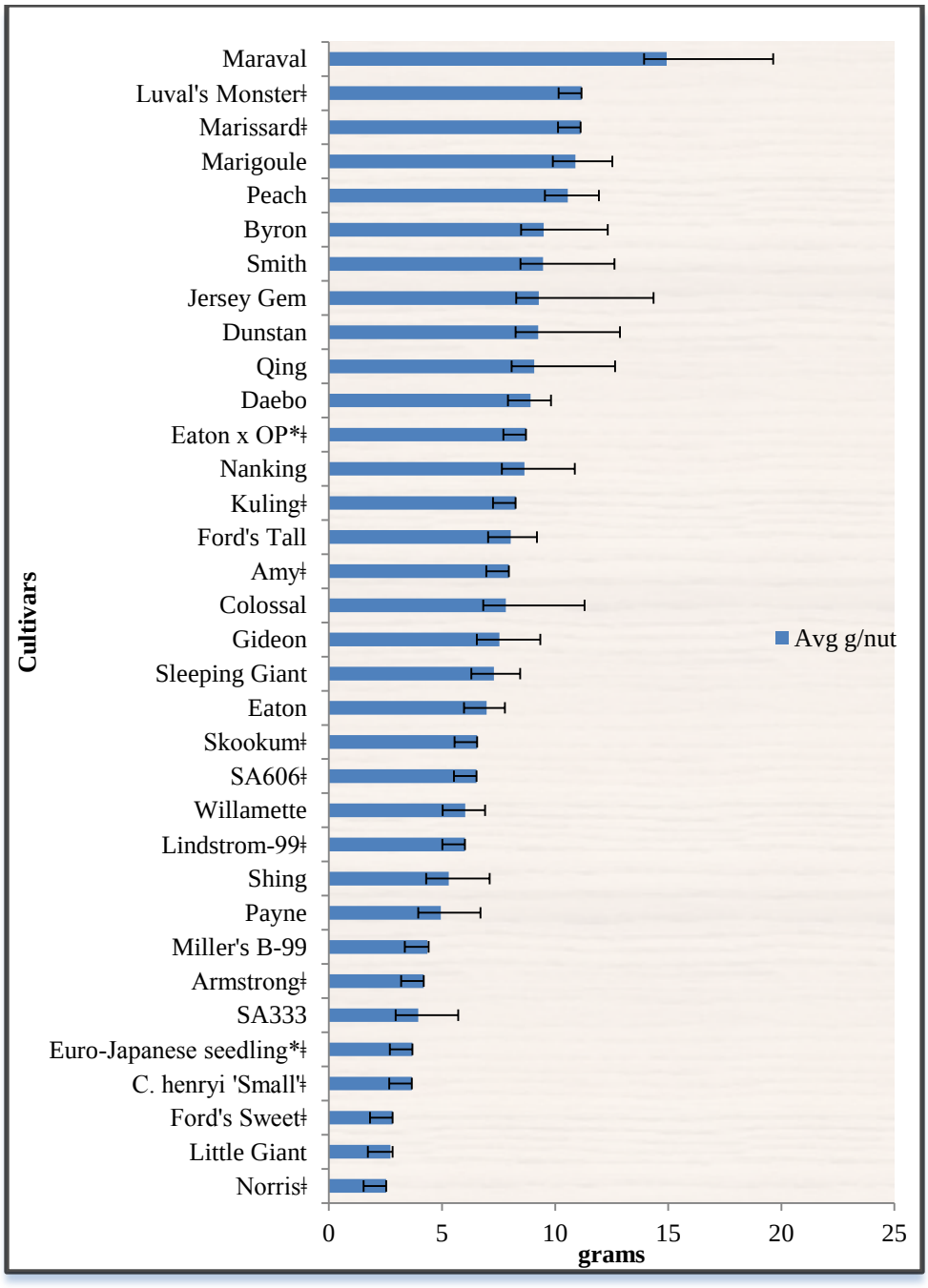

Figure 6 Average grams/nut for 34 chestnut cultivars and *seedling rootstocks in 2010; ‡one accession harvested; error bars represent one standard deviation from the mean

‘Marigoule’ (2.81 kg/tree) yielded significantly more kilograms of fruit than ‘Little Giant’ (0.373 kg/tree), ‘Sleeping Giant’ (0.235 kg/tree), and ‘Byron’ (0.188 kg/tree), but not more than the other cultivars in 2010 (Table 9). ‘Colossal’ (3.21 kg/tree) had larger yields than ‘Sleeping Giant’ (0.235 kg/tree) and ‘Byron’ (0.188 kg/tree), but not more than the other cultivars. ‘Maraval’ (0.968 kg/tree), ‘Qing’ (1.66 kg/tree), ‘Payne’ (0.780 kg/tree), ‘Shing’ (0.945 kg/tree), ‘Dunstan’ seedling (0.834 kg/tree), ‘Gideon’ (0.553 kg/tree), ‘Nanking’ (0.626 kg/tree), ‘Willamette’ (0.501 kg/tree), ‘Eaton’ (1.28 kg/tree), ‘SA333’ (0.470 kg/tree), and ‘Peach’ (0.656 kg/tree) were equivalent in yields.

Table 9 Results of Tukey’s Studentized Range (HSD) Test for 2010 cultivar yield (kg per tree)

Cultivar	**N Harvested**	**Tukey Grouping***	**Avg. kg/tree ± SD**
Marigoule	4	a	2.81 ± 1.12
Colossal	9	a, b	3.21 ± 1.97
Maraval	5	a, b, c	0.96 ± 0.254
Qing	6	a, b, c	1.66 ± 1.34
Payne	6	a, b, c	0.78 ± 0.418
Shing	10	a, b, c	0.94 ± 0.724
Dunstan seedling	6	a, b, c	0.83 ± 0.631
Gideon	13	a, b, c	0.55 ± 0.244
Nanking	4	a, b, c	0.62 ± 0.450
Willamette	3	a, b, c	0.50 ± 0.134
Eaton	3	a, b, c	1.28 ± 1.57
SA333	9	a, b, c	0.47 ± 0.315
Peach	3	a, b, c	0.65 ± 0.726
Little Giant	3	b, c	0.37 ± 0.153
Sleeping Giant	5	c	0.23 ± 0.135
Byron	10	c	0.18 ± 0.166
Total 16 cultivars	**99**		

*Means with the same letter are not significantly different ($p \geq 0.05$).

‘Maraval’ had a high outlier tree in the harvest of 2010 with a yield of 1.41 kg (unranked data) out of the five trees harvested. ‘Colossal’ had an extremely low outlier tree in 2010 of only 0.06 kg (ranked data); and ‘Gideon’ had a low outlier tree of 0.101 kg (ranked data). The harvests of all cultivars in 2010 including those that may have had less than three trees per cultivar are shown in Figure 6.

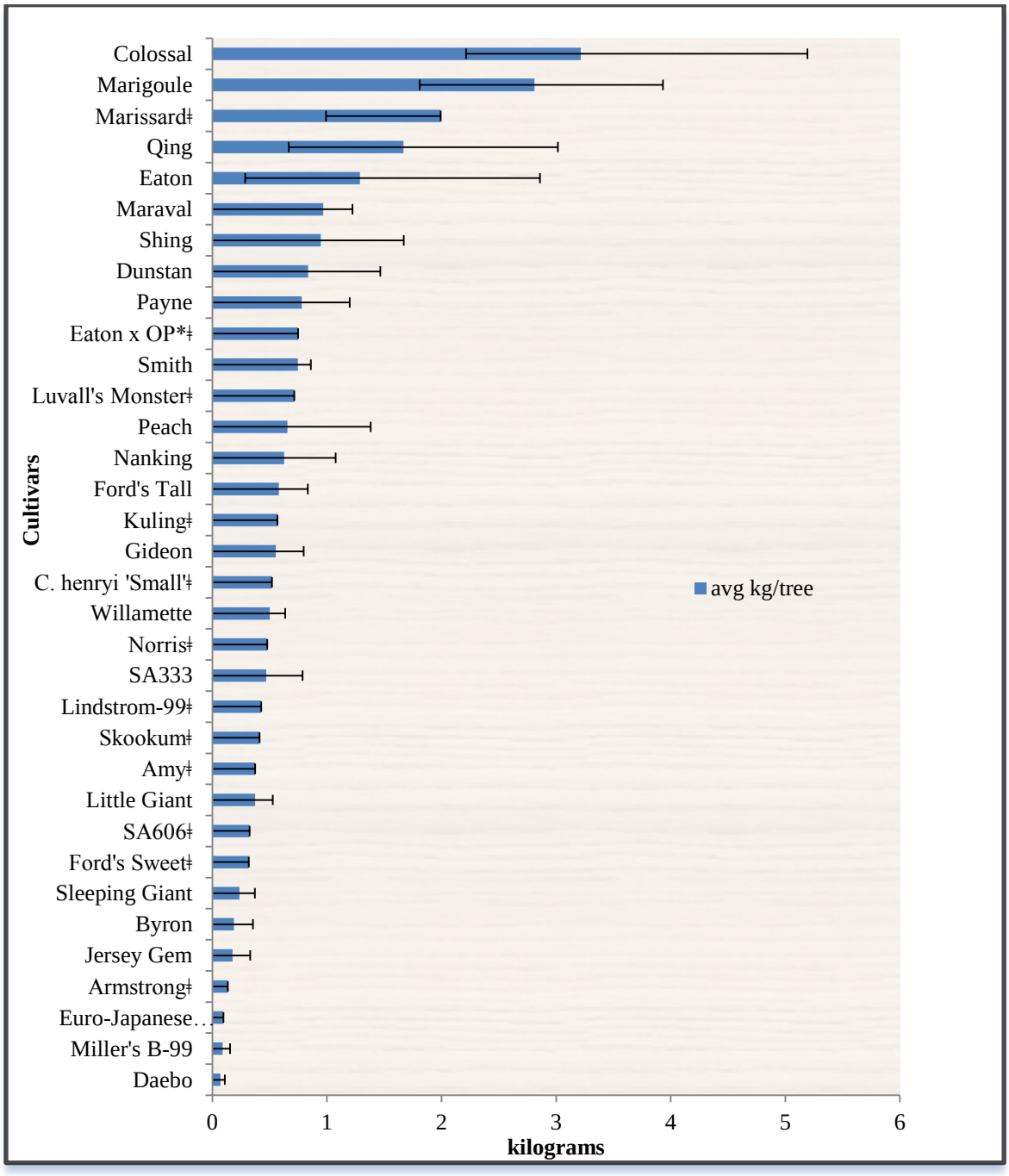

Figure 7 Average yield (kg/tree) for 34 chestnut cultivars and *seedling rootstocks in 2010; ǂone accession harvested; error bars represent one standard deviation from the mean

2011 nut mass and yield

We harvested from 15 cultivars in 2011, but performed data analysis only on 11 cultivars for which three or more trees were harvested in 2011. Year 2011 was neither productive in yields nor nut size (Fig. 7 and 8). The only significant differences observed in 2011 in nut mass were between 'Little Giant' (3.880 g/nut) and the cultivars 'Eaton' (10.67 g/nut), 'Eaton River' (8.439 g/nut), 'Nanking' (9.925 g/nut), and 'Colossal' (8.878 g/nut) in that 'Little Giant' was smaller than those cultivars in size. However, it was not different from 'Qing' (6.451 g/nut), 'Sleeping Giant' (6.598 g/nut), 'Gideon' (5.487 g/nut), 'SA333' (5.467 g/nut), 'Amy' (5.810 g/nut), and 'Shing' (4.505 g/nut). All of the other 11 cultivars harvested were similar to each other (see Table 10).

Table 10 Results of Tukey's Studentized Range (HSD) Test for 2011 cultivar nut mass (grams/nut)

Cultivar	N Harvested	Tukey Grouping*	Avg. g/nut ± SD
Eaton	5	a	10.6 ± 4.98
Eaton River	5	a	8.43 ± 0.969
Nanking	6	a	9.92 ± 4.25
Colossal	4	a	8.87 ± 2.27
Qing	6	a, b	6.45 ± 1.75
Sleeping Giant	3	a, b	6.59 ± 1.57
Gideon	6	a, b	5.48 ± 1.64
SA333	6	a, b	5.46 ± 1.11
Amy	4	a, b	5.81 ± 1.30
Shing	6	a, b	4.50 ± 0.975
Little Giant	4	b	3.88 ± 0.257
Total 11 cultivars	**55**		

*Means with the same letter are not significantly different ($p \geq 0.05$).

No significant differences in yield ($p > 0.05$) were detected between cultivars in 2011 (Table 11). However, 'Gideon' had an extreme outlier tree (both ranked and unranked data) that yielded 7.45 kilograms, a particularly high yield considering the generally poor yield of all trees in 2011. Harvest in 2011 for all cultivars' nut mass is represented graphically in Fig. 7 and 8.

Table 11 Results of Tukey's Studentized Range (HSD) Test for 2011 cultivar yield (kg per tree)

Cultivar	N Harvested	Tukey Grouping*	Avg. kg/tree ± SD
Amy	4	a	1.41 ± 0.461
Colossal	4	a	1.96 ± 1.34
Shing	6	a	1.17 ± 0.574
Eaton	5	a	1.19 ± 0.670
Qing	6	a	1.18 ± 1.07
Gideon	6	a	1.85 ± 2.80
SA333	6	a	0.60 ± 0.301
Nanking	6	a	0.49 ± 0.379
Sleeping Giant	3	a	0.40 ± 0.274
Eaton River	5	a	0.32 ± 0.127
Little Giant	4	a	0.21 ± 0.113
Total 11 cultivars	**55**		

*Means with the same letter are not significantly different($p \geq 0.05$).

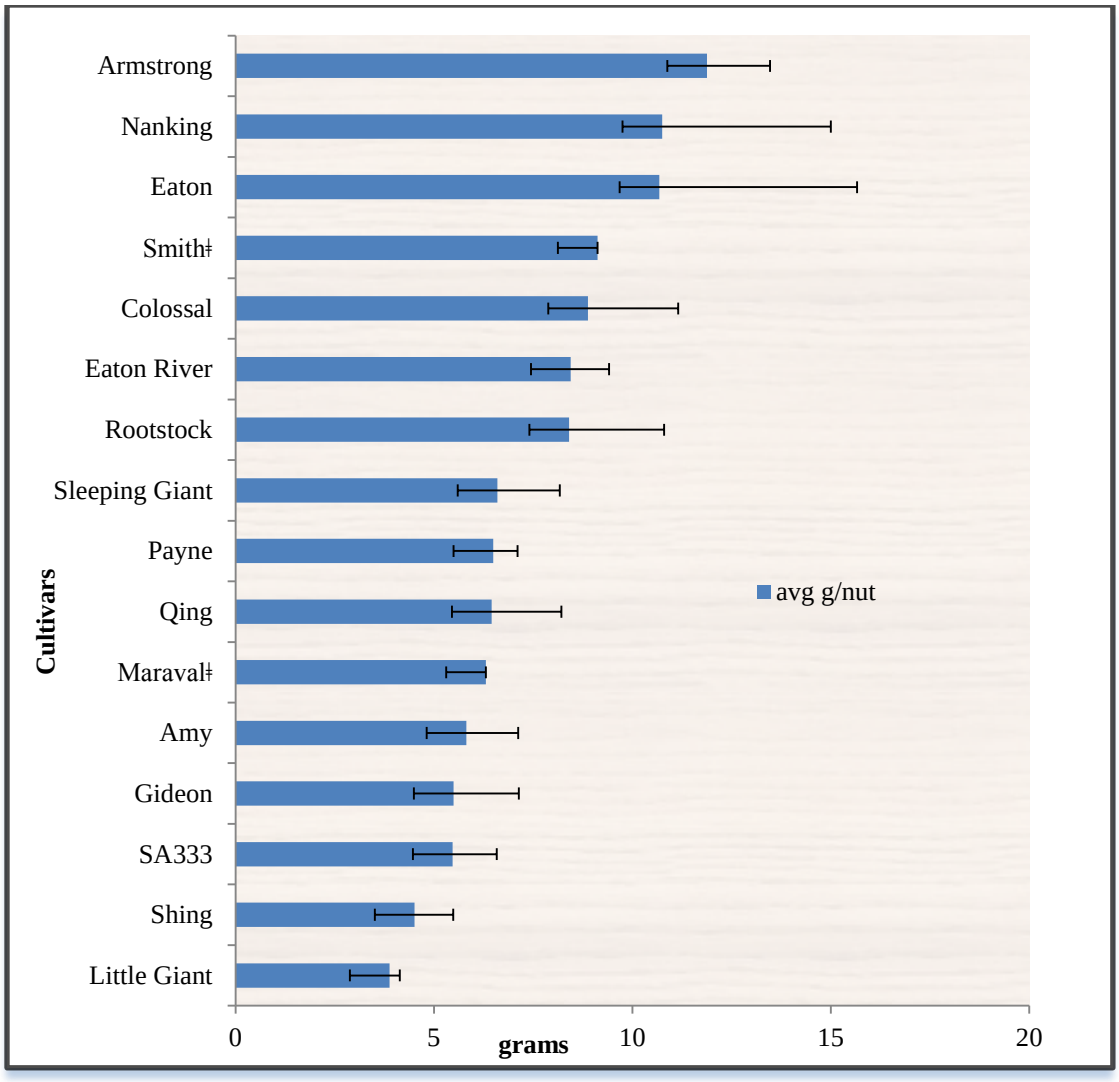

Figure 8 Average grams/nut for 15 chestnut cultivars in 2011; ‡one accession harvested; error bars represent one standard deviation from the mean

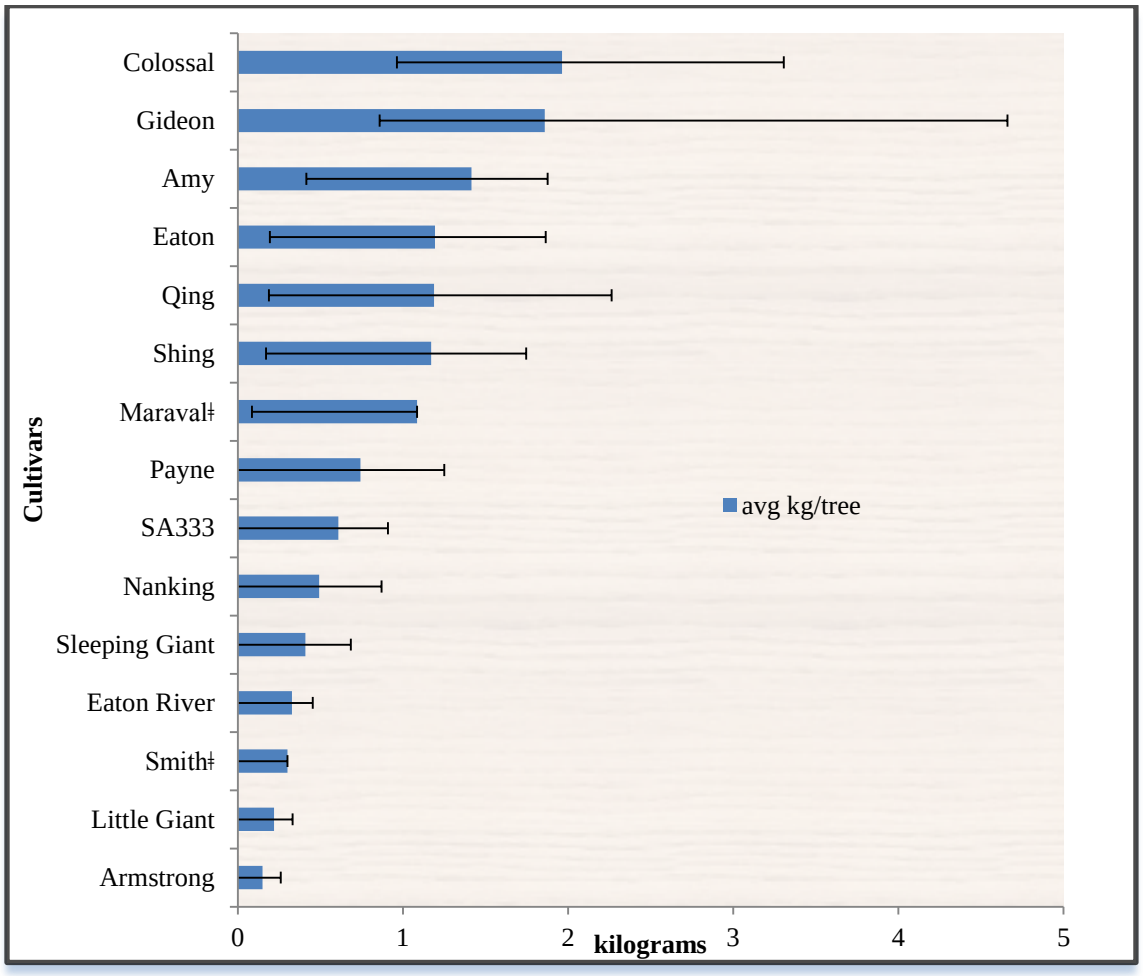

Figure 9 Average yield (kg/tree) for 15 chestnut cultivars in 2011; ‡one accession harvested; error bars represent one standard deviation from the mean

2008-2011 nut mass and yield

Average nut mass and yield for all years 2008-2011 show significant overlap, both within each year and in all years combined. The only significant difference in nut size observed was between 'Marigoule' (18.3 g/nut), 'Amy' (7.16 g/nut), 'SA333' (4.55 g/nut), 'Little Giant' (4.34 g/nut), and 'Maraval' (13.4 g/nut), where 'Marigoule' (18.3 g/nut) was significantly larger in nut mass. 'Maraval' (13.4 g/nut) was only significantly larger than 'Little Giant' (4.34 g/nut). No other significant differences between cultivars were observed for the combined years 2008-2011 (Table 12). The nut weights of cultivars in the individual years 2008-2011 are shown in Figure 9, while the nut weights of all cultivars for combined years 2008-2011 are shown in Figure 10. The total average yields per cultivar for all years are shown in Figure 11.

Table 12 Results of Tukey's Studentized Range (HSD) Test for cultivar nut mass (grams/nut) for years 2008-2011

Cultivar	N Harvested*	Tukey Grouping**	Avg. g/nut ± SD
Marigoule	14	a	18.3 ± 5.78
Colossal	27	a, b	15.8 ± 7.67
Peach	9	a, b	14.6 ± 3.64
Marissard	3	a, b	18.5 ± 11.2
Maraval	6	a, b, c	13.4 ± 5.37
Eaton River	10	a, b, c, d	11.8 ± 3.41
Eaton	12	a, b, c, d	11.9 ± 4.55
Byron	20	a, b, c, d	11.6 ± 3.68
Nanking	19	a, b, c, d	11.9 ± 4.32
Qing	20	a, b, c, d	11.6 ± 5.04
Norris	3	a, b, c, d	11.1 ± 6.12
Willamette	5	a, b, c, d	12.5 ± 8.04
Gideon	42	a, b, c, d	11.0 ± 4.52
Dunstan seedling	12	a, b, c, d	10.7 ± 3.98
Fords Tall	5	a, b, c, d	10.2 ± 2.16
Payne	24	a, b, c, d	10.2 ± 4.52
Skookum	3	a, b, c, d	9.61 ± 2.22
Shing	28	a, b, c, d	10.5 ± 7.13
Jersey Gem	4	a, b, c, d	10.1 ± 5.38
Sleeping Giant	16	a, b, c, d	9.56 ± 3.38
Smith	3	a, b, c, d	9.35 ± 2.57
Armstrong	4	a, b, c, d	9.20 ± 3.17
Amy	5	b, c, d	7.16 ± 2.58
SA333	15	c, d	4.55 ± 1.70
Little Giant	10	d	4.34 ± 1.74

*Number harvested does not represent the total number of trees of the cultivar in the orchard. Number harvested is the cumulative number of trees of the cultivar harvested from years 2008-2011. Statistical data analysis was only performed on those cultivars that had at least three harvests from them within the years 2008-2011.
**Means with the same letter are not significantly different ($p \geq 0.05$).

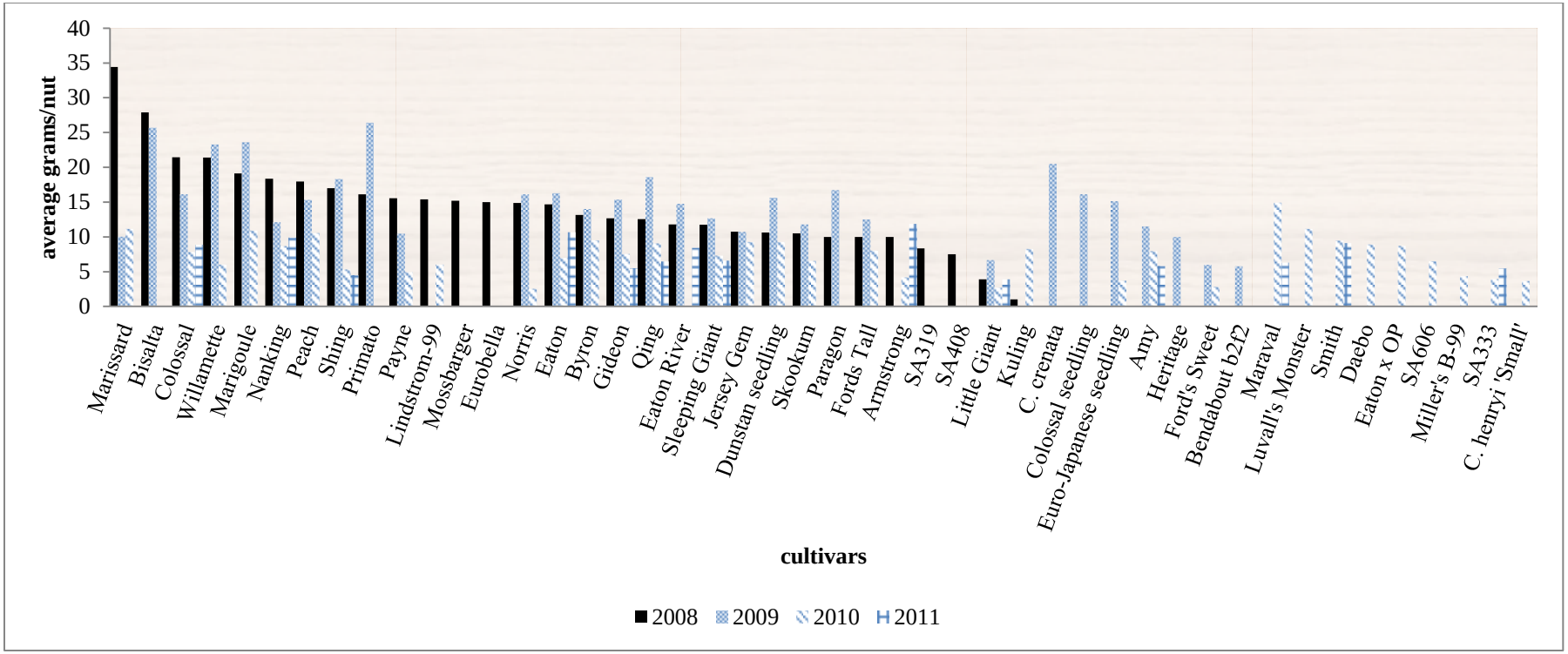

Figure 10 Average grams/nut for 46 chestnut cultivars from 2008-2011

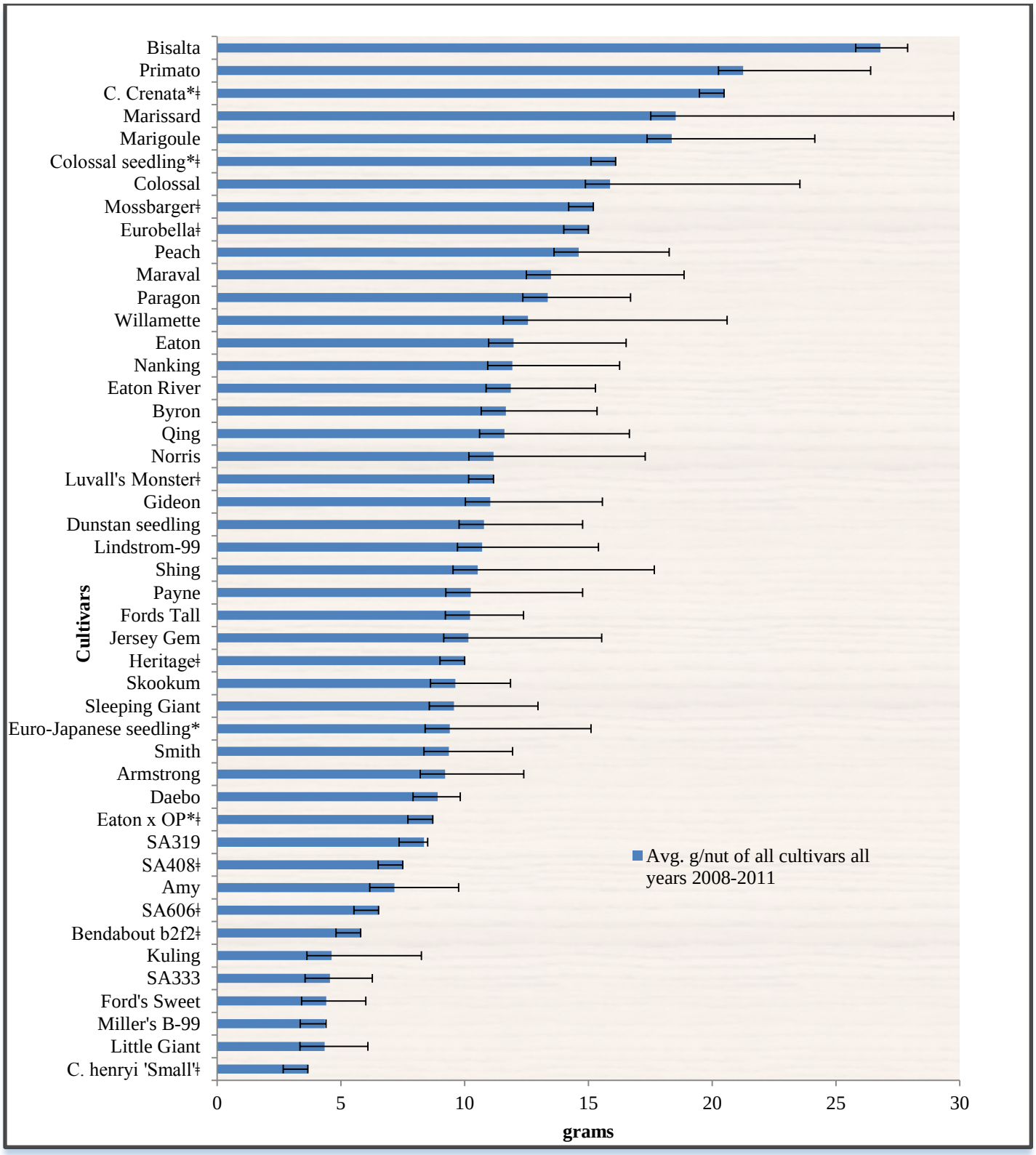

Figure 11 Average grams/nut for 46 chestnut cultivars and *seedling rootstocks for combined years 2008-2011; ǂone accession harvested; error bars represent one standard deviation from the mean

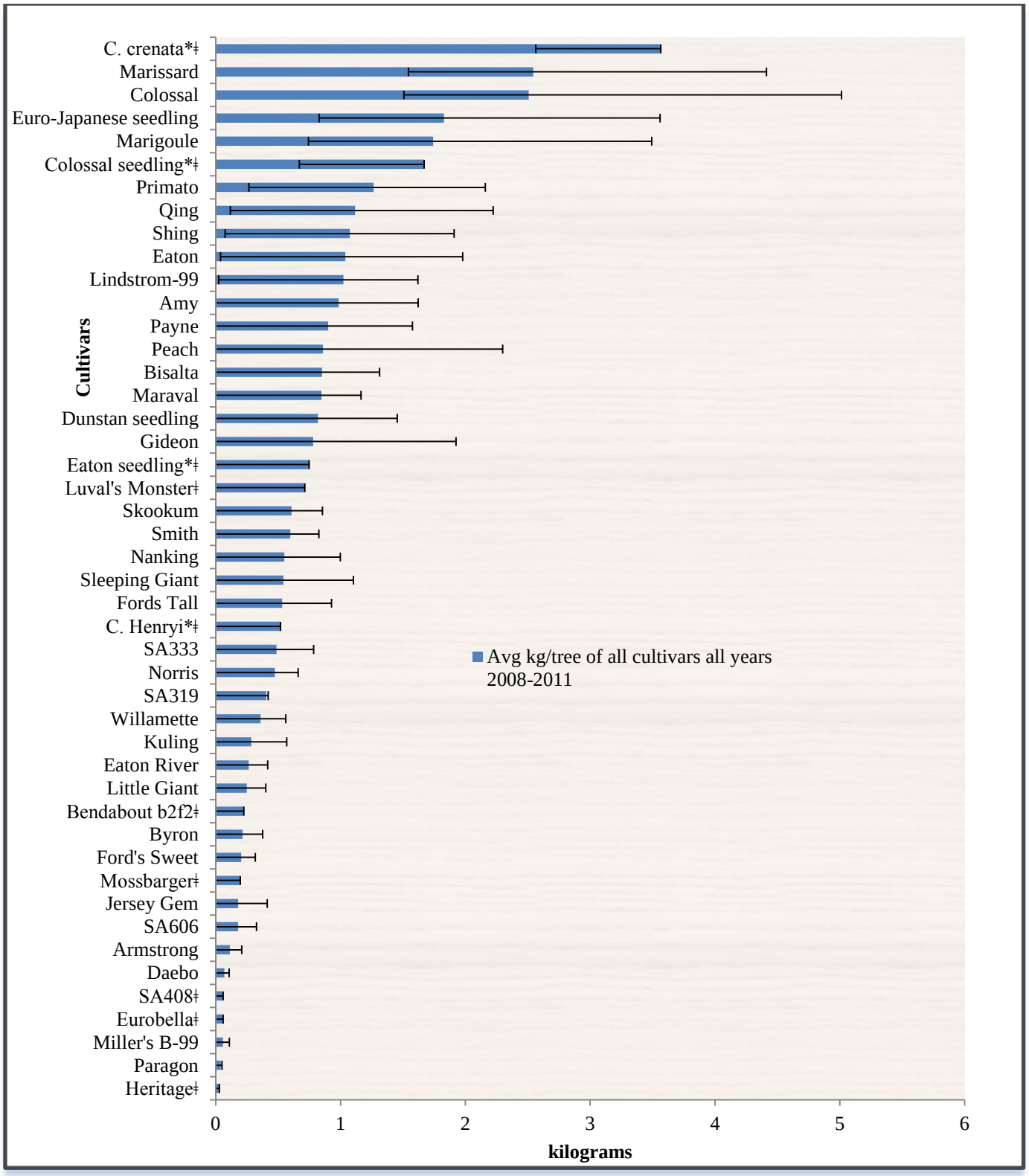

Figure 12 Average kg/tree for 46 chestnut cultivars and *seedling rootstocks for combined years 2008-2011; ǂone accession harvested; error bars represent one standard deviation from the mean

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Based on the results for nut mass and yield per cultivar for years 2008-2011, no particular cultivars are strongly favored. The results presented here, although they are based on more than four years of observation, must be labeled preliminary, since they are based on small samples of relatively young trees of a limited number of accessions; but the results expose some potential chestnut cultivar candidates for a commercial chestnut orchard in Tennessee.

The orchard has received many setbacks that have restricted the original structure of the study and postponed net profit from investments into the orchard, including ambrosia beetle (Coleoptera: Scolytidae), Japanese beetle (Coleoptera: Scarabaeidae), and possibly other insect damage, and, most catastrophically, a late spring freeze in 2007, which significantly reduced the number of tree accessions and resulted in substantial graft failure. In 2007, drought occurred that was among the most severe in Tennessee history, which led to decreased yields in many agricultural commodities and even had urban impacts (Goodrich et al., 2011).

Adequate water throughout the growing season was not available every year. Most of the trees at Smith Farm survived during droughts but the nuts were of such poor quality that many were unsellable, which would result in a significant profit loss. The same conclusion of drought negatively affecting harvests and therefore profits was drawn by Hunt et al. (1998). 'SA333' and 'Shing' in particular manifested drought stress in 2010 by dropping the burrs with the nuts strongly attached and difficult to pry loose, and the cotyledons were dry and shriveled. There were droughts just before harvest season in 2010 and 2011. Years 2010 and 2011 produced poorer quality, small nuts, than the years 2008 and 2009 even in cultivars that had generally produced large nuts.

In 2010, total seasonal rainfall and the majority of months from June-August were 11.1 cm below normal in Chattanooga, with a total rainfall in this period of 20.0 cm and the 17th driest summer on record (Glenn, 2010). This was a critical period just before harvest, and the orchard was not irrigated. Also, Summer 2010 ranked as the warmest average seasonal temperature in Chattanooga with an average of 28ºC, which was warmer than the last

record in 1993 with an average seasonal temperature of 27ºC. The average temperatures in August also were above normal, the second warmest August on record (Glenn, 2010).

The 2011 harvest also may have been compromised by inadequate rainfall in August: 0.025 cm, compared with 7.74 cm in August 2010 and 7.56 cm in August 2009. September 2010 was a record month and 2010 was a record year for drought in Chattanooga according to the Southeast Regional Climate Center, having an average rainfall in September of 2.87 cm, compared to 31.4 cm in 2009 (SERCC, 2012). This result corresponds to the data provided by the study of Borghetti et al. (1986). This period before harvest seems to be critical for fruit growth (Borghetti et al., 1986).

From 2008-2011, 'Colossal' and 'Marigoule' were in the top five for largest average nut size three times, 'Marissard', 'Bisalta', and 'Willamette' twice, and 'Maraval', 'Luvall's Monster', 'Peach', 'Armstrong', 'Eaton', 'Nanking', 'Smith', and 'Primato' once. In this same time period, 'Colossal' was in the top five for greatest average yield in kilograms per tree four out of five times, 'Qing' three times, 'Marissard', 'Marigoule', and 'Eaton' twice, and 'Primato', 'Shing', 'Lindstrom-99', *C. crenata* seedling, 'Gideon', 'Amy', and a Euro-Japanese seedling once. Based on these facts, a grower might intuitively select 'Colossal', 'Marigoule', and 'Marissard', and derive moderate benefit in both fruit size and yield.

'Colossal' ranked second in nut size in 2009, first along with 'Nanking' in 2008, and first along with 'Eaton', 'Eaton River', and 'Nanking' in 2011. However, its blight susceptibility, although moderate, and gradual visible decline once it acquires *Cryphonectria parasitica*, makes dependence on it for income unadvisable. 'Marigoule' nuts, despite ranking second for nut size in 2008, first in 2009, and second in 2010, manifested poor nut quality in that they were difficult to peel and were among the first to spoil in refrigerated storage. We have only one 'Marissard' tree, which was harvested each year from 2008-2011. This renders making a definitive statistical statement about the possibility of it having large nuts difficult, as 'Marissard' could not be included in the data analysis in each year. However, we were able to include 'Marissard' in the cumulative data analysis of 2008-2011 and we found that 'Marissard' was amongst the highest ranking cultivars in nut size with an average of 18.5 g/nut for all years. 'Little Giant' consistently ranked smallest among all cultivars from 2008-2011, and thus we do not encourage its planting in Tennessee. None of the cultivars with European pedigrees fared well. Having European or hybrids with European fruit characteristics, such as monoembryony, free-peeling pellicles, and raised stripes, that

were tolerant of late spring freezes and were blight-resistant would be very desirable. If such cultivars exist, they were not apparent in this experiment.

'Gideon' was never among the largest chestnuts, but it was never among the smallest either. One of the reasons that 'Gideon' may be recommended for growing in Tennessee is that in the absolutely worst harvest year of 2011 for fruit size and yield, one of the five 'Gideon' trees harvested was an extremely high outlier with a yield of 7.45 kilograms (unranked data). This was the tree that yielded the highest mass of fruits for 2011 of those that were statistically analyzed. The next heaviest yield in that ear was from a 'Colossal' tree that bore 3.773 kg.

Although 'Sleeping Giant' and 'Byron' did not significantly differ from 12 out of the 14 other cultivars analyzed in 2010 (Table 10), they still ranked at the very bottom in yield per tree in that year. Also, 'Byron' produces a very odd-shaped, three-sided nut which may not be commercially valuable because it may be perceived as unattractive. However, an interesting quality of 'Byron' is that it holds its branches upright (fastigate). This characteristic may be of value in future breeding programs, as branch crowding can become problematic in mature orchards. Furthermore, 16 out of the original 20 of the 'Byron' trees have survived so far, which is amongst the best survivorship of all cultivars. It has obviously withstood the continued presence of *Cryphonectria parasitica*, ambrosia beetles, and late spring freezes.

Average nut mass and yield for all cultivars all years 2008-2011

The cultivars that produced the absolute largest average nuts throughout the years 2008-2011 ('Bisalta', 'Primato', *C. crenata* seedlings, 'Marigoule', 'Colossal', 'Mossbarger', 'Eurobella', 'Peach;, 'Paragon', and 'Willamette') are not necessarily the cultivars a grower might wish to plant in Tennessee. 'Bisalta' was only harvested twice from 2008-2011 (Figure 9), so it was difficult to make an accurate assessment from two data points. 'Primato' also was harvested twice, but then the only accession died.

Some of the cultivars in the germplasm collection such as 'Maraval' (13.4 g/nut) stored well, but they were difficult to peel. Although no formal peel-ability and storability analyses were undertaken, we did peel several of our cultivars by hand and noted which cultivars' nuts stored particularly poorly. 'Marissard' peels well (Nave, 1998), but we only have one accession, which was harvested three times. It was one of the cultivars in the germplasm collection and could be worth growing based on its large (13.49 g/nut) attractive nuts. 'Paragon' (13.35 g/nut) would be a very desirable tree to grow based on its historical record of large, appealing nuts; but it is blight-susceptible

(Sober, 2009). We harvested the single 'Eurobella' and thus we cannot make a strong argument for it based on one tree; though it might be worth growing more of it since it is easy to peel and a good pollenizer (Grunder, 2007).

It is too risky to grow 'Colossal' and 'Colossal' seedlings in Tennessee because they are blight susceptible (Anagnostakis, 2012). There was only one harvest from 'Mossbarger' and there are only three accessions left as of 2012 from the original 20 in the randomized complete block experimental design. Most of the 20 died in the 2007 late spring freeze, so it likely has a difficult time surviving in an area prone to late spring frosts/freezes. 'Peach' (14.6 g/nut) also had very poor survival. There are only six left out of the original 20, which was contrary to our expectations as it was recommended by Greg Miller, a successful chestnut grower in Ohio (Miller, 2012). 'Willamette' (12.5 g/nut) was one of the cultivars in the original randomized complete block experimental design of which we originally had 20 but now only have four. Thus, it appears not to survive well in late-spring frost areas. We expected it to do well because it is a blight-resistant, easy-to-peel chestnut patented by R.D. Wallace (Wallace, 1990); but our results do not confirm this hypothesis. The cultivars we do recommend, 'Eaton' (11.9 g/nut), 'Nanking', (11.9 g/nut), 'Eaton River' (11.8 g/nut), 'Qing' (11.6 g/nut), 'Gideon' (11.0 g/nut), 'Shing' (10.5 g/nut), 'Payne' (10.2 g/nut), and possibly 'Lindstrom-99' (10.7 g/nut), may not necessarily show the largest nuts when averaged over the years 2008-2011 (Figure 10), but that is mostly due to the years 2010-2011 being extreme drought years that skewed the data. Further studies might examine these cultivars in a similar area but managed with an irrigation system.

Similarly, we also cannot reject or recommend certain cultivars based purely on their total average yield from 2008-2011 (Fig. 11). A major reason for this is that not every nut was picked every year due to a limited number of volunteers. *Castanea crenata* had the highest average kilograms (3.56 kg/tree) for all the years 2008-2011; but that is an average based on one harvest datum. Still, a few *C. crenata* seedlings in an orchard in Tennessee would increase the diversity of the germplasm in the orchard, which we encourage for sustainability. 'Marissard' had the second highest average (2.54 kg/tree), but we have only one accession and it was difficult to draw conclusions from a sample of one. Also, it often bore fruit so heavily that its branches broke. We nonetheless think that it is a high potential candidate because it was heavy-bearing and produced large, attractive nuts (18.5 g/nut). 'Colossal' was the third highest yielding (2.50 kg/tree) for all years 2008-2011, and that was based on an average of 38 data points; but, again, it is too blight susceptible to be a low-risk, profitable cultivar.

‘Marigoule’ was a higher-yielding cultivar (1.74 kg/tree) and produced large nuts (18.3 g/nut) so it is desirable in those respects. Plus, it is *Phytophthora*-resistant (Nave, 1998). However, we caution that it should probably be stored in a refrigerator that is capable of storing chestnuts at an optimal temperature range of -6° to 2°C (Payne et al., 1982) or below 0°C (Jermini et al., 2006) because our ‘Marigoule’ nuts did not store well. ‘Maraval’, a Euro-Japanese hybrid from France, has nuts that store well and yielded an average of 0.846 kg/tree. In respect to yield and nut size (13.4 g/nut) was acceptable.

Based on our results on the cultivars’ yields, ‘Qing’, ‘Shing’, ‘Eaton’, ‘Gideon’, ‘Nanking’, and ‘Eaton River’ do not appear to be productive, but their low average yields may be explained as a reflection of a lack of water availability. Therefore, we suggest them as candidates for chestnut cultivation in Tennessee based on the 2008-2009 results.

Periods of low rainfall can be mitigated by an irrigation system, and deer browsing can be prevented by fencing. Gall wasp can be combatted by following quarantine protocols of plant material, or if necessary, introducing the parasitoid *T. sinensis*. The continual presence of chestnut blight can be dealt with by planting blight-resistant cultivars or treating susceptible cultivars periodically with a hypovirulent strain of *C. parasitica*, although the latter solution may be more difficult to accomplish due to the potential for vegetative incompatibility (Robin and Heiniger, 2001) and the expense of maintaining such an operation. However, even if all of the above conditions were met, the risk of late spring frosts and freezes in the Southeast will be a perennial issue for commercial chestnut production (Wang and Fu, 2010; Lianhong et al., 2008). Agricultural consequences of the effects of frosts, particularly in the context of climate change, could be the subject of future studies. The distribution and frequency of frost events may potentially change and become more common as an effect of global climate change (Inouye, 2000).

Based on the fact that *C. mollissima* has significantly higher genetic variability than all other *Castanea* species (Huang et al., 1994c), I conclude that Chinese chestnut cultivars might have a higher likelihood of late spring frost tolerance. Furthermore, *C. mollissima* is more likely to be chestnut blight, ink-disease, and if such a trait exists, gall-wasp resistant (Maynard et al., 2008; Ding et al., 2004). Therefore, I recommend that any commercial chestnut orchard in Tennessee be established on *C. mollissima* stock. From the commercial standpoint of production, Chinese chestnut seedlings can exhibit a wider range of characteristics than seedlings of other species (Moore,

1948). However, it should be pointed out that our *C. dentata* and The American Chestnut Foundation's *C. dentata* hybrids were not affected by the 2007 freeze.

One solution that obviates the need to conclusively resolve these perplexities is to plant most of the orchard with cultivars, and some of the orchard with seedlings. Gradually rogue both the seedlings and the cultivars, until eventually nearly every tree in the orchard is a commercially remunerative tree in some respect, whether it is a chestnut tree that produces fruits most ideal for fresh consumption, or a chestnut tree that produces fruits better suited for value-added, processed chestnut products. In the meantime, while this optimal cultivar to seedling ratio is being developed, the seedlings can serve as pollenizers for any pollen-sterile cultivars. If a seedling proves to be worthless for chestnut production in and of itself, it can either be grafted with a named cultivar or replaced with another seedling. This system may be capable of resolving much of the dilemma between planting seedlings and cultivars.

Similar suggestions have been put forth in the Pacific Northwest where chestnut blight is absent. In one variation of this proposal, a grower can interplant an alternate, blight-resistant tree with every other tree being a productive, though blight-susceptible, cultivar with desirable nut qualities. Then, if chestnut blight were to become persistent in the Pacific Northwest, the alternate trees would continue the orchard business (Rackham, 1987). But with chestnut blight already being prevalent in the southeastern United States, there is a more urgent need to start off with mostly blight-resistant cultivars along with some seedlings. After enough time, the seedlings that do not readily succumb to late spring frosts and freezes will become apparent.

Another reason to attempt to establish a commercial orchard partially based on Chinese seedlings is that, even if a grower has a fairly certain idea of the best possible cultivar for a given situation, choices may be limited by cultivar availability in the nursery trade (Craddock, 2006). This finite accessibility also can restrict a cultivar field test's statistical power. In some cases, it is necessary to perform experimentation on a cultivar represented by only one tree, which may be due to other clones having died, or, it may be an unusual cultivar (Martin et al., 2009). This situation occurred at our experimental orchard in several cases. Thus, caution is necessary before certain cultivars can be completely dismissed or others commended, leaving the question of which ones to plant partially unresolved.

A mature chestnut orchard with reliable grafted cultivars can annually yield 224 kg per hectare (Warmund, 2011). However, as important as yields is the appearance of chestnuts that will be marketed for fresh consumption (Solar et al., 2005). Cultivars' nuts were visually observed as they were harvested, counted, and weighed. Our

'Gideon' showed the desirable characteristic of a bright pericarp with darker stripes, which is indicative of high-quality chestnuts (Solar et al., 2005). 'Gideon' also generally bore larger-sized nuts and were easy to peel.

Another element of a chestnut orchard, the importance of which may become more apparent in the development of an orchard, is the rootstock. A reason to predict this is that they are important in other fruit production industries. For example one of the most critical components impacting the profitability of an apple orchard are the rootstocks, as they can influence tree vigor, yield, mortality, and fruit size. But rootstock performance also varies based on location (Marini et al., 2006). Even if cultivar clones, as opposed to seedlings, dominate an orchard in Tennessee, we also recommend that the rootstocks be primarily based on *C. mollissima*.

Despite the range of difficulties in a commercial chestnut orchard that have been addressed throughout this study, the results obtained suggest that there is potential for commercial chestnut orchards to be successful in the southeastern United States. On another level, this study underlines the compromise between what can be expected from the potential resources of chestnut orchards, the economic demand of those resources, and the different factors and participants implicated on a local level. The extent of economic viability of a chestnut orchard is strongly linked to a variety of factors, including weather and climate conditions, presence of pests and diseases, financial inputs into the orchards, time spent on maintenance, farmer expertise, quality of the land, availability of marketing outlets, mechanized equipment, market demand, and the significance of the agricultural enterprise to the investors.

There is potential for commercially viable chestnut orchards in Hamilton County, Tennessee. However, climatic conditions and potential pests and diseases in the area render such an enterprise less ideal and more problematic than conditions in Oregon, California, and Michigan (Craddock, 2006). Other difficulties are Tennessee's marketing channels the area's lack of access to mechanization (harvesting and processing). Positive aspects of chestnut orchards in general are that there is a progressive decrease in terms of work (Conedera et al., 2001), a strong public demand for chestnuts (Aguilar et al., 2009), and their intrinsic ecological value[1] (Conedera et al., 2001). If resources are available for an initial investment and if the maintenance of chestnut orchards can be paid for with the profits made from their products, commercial chestnut production in Tennessee is possible and capable of sustaining itself.

[1] The Cévennes sweet chestnut forests in Languedoc-Roussillon are considered to be rural or domestic forests, which are managed by farmers as agroecosystems as opposed to conventionally managed forests. Large contiguous stands of grafted *C. sativa*, likely a relic of the Roman Empire, dominate certain forested areas in the Cévennes (Aumeeruddy-Thomas et al., 2012).

Many chestnut groves in Europe that are hundreds of years old can be viewed as part natural/part anthropogenically-influenced ecosystems, considering that they involve plant associations in which other flora have been changed considerably (Arnaud et al., 1997). The groves' survival often depends on both the care given to their cultivation and the ecological conditions of the area. Fortunately for the chestnut grower, most chestnut farming systems lead to a gradual decrease in terms of work. That is, as the orchard matures, necessary agricultural maintenance decreases (Arnaud et al., 1997).

If the chestnut producer is either a part-time farmer or diversifying an existing agricultural operation, as many are in the United States (Gold et al., 2005), then increasing agricultural inputs into chestnut tree-growing may not appear to be financially justifiable. Although the investment in a small-scale operation is initially low (Gold et al., 2005) and chestnut orchards can be managed with relatively little maintenance, a new plantation may take up to 15 years to net profit. This break-even time-period for chestnut orchard investment is so long, that adopting suboptimal orchard management practices (e.g. operating without an irrigation system or deer fence) may delay profits even further. Happily, more effective orchard management regimes are not really significantly more complex or time-consuming than suboptimal management practices, and small improvements in the orchard system should lead to significantly higher yields and profits (McLaren, 1999).

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APPENDIX A

CASTANEA PALEOBOTANY

The genus *Castanea* appears in the fossil record around 30-65 million years ago (mya) in the early Paleogene period (Taylor, 2009). During the Paleocene (≈61.1 mya), the family Castaneoideae underwent a major radiation. *Castanea* had colonized the northern hemisphere around 40 mya, before Europe and North America had separated. *Castanea* was a keystone genus by the Neogene (≈23 mya), and was abundant and persistent in N. America, east Asia, and Europe at the end of the Pleistocene epoch (11,700 years ago) and the beginning of the Holocene epoch (since 10,000 years ago to the present) (Krebs et al., 2004).

Castanea was more prevalent in the Tertiary than it is today (Lang et al., 2007). Macrofossils of *Castanea* dating from the Paleocene to the early Eocene (40 mya) have been uncovered in the Rocky Mountains of North America (Lang et al., 2007); and Castaneoid inflorescences have been reported from the middle Eocene in Tennessee (Taylor et al., 2009). Fossilized leaves and cupules dated to the Eocene that closely resemble modern *Castanea* species suggest that the genus has had a long-term presence in eastern North America (Manos and Stanford, 2001). *Castanea* fossils have also been found in eastern Asia from the late Eocene to the Pliocene, in Japan from the Oligocene (33.9 mya), and in North Korea from the Miocene (23-3.6 mya) (Lang et al. 2007).

Castanea pollination

Pollination in *Castanea* is generally anemophilous (wind-pollinated); but, it has also been described as entomophilous (insect-pollinated), as well as a combination of both (Hardin et al., 2001). The anemophilous syndrome is generally characterized by flowering before leafing out, copious amounts of lightweight pollen, inconspicuous pistillate flowers, a lack of nectar and odorless pollen. The entomophilous syndrome is normally expressed by leafing out before flowering, heavier, sticky pollen in fewer amounts, and aromatic, showy flowers (Hardin et al., 2001). *Castanea* leafs out before flowering, its pollen grains are relatively small (Paillet, 2002), odiferous and sticky (Paillet et al., 1991); and it is apetalous and produces abundant yellow-ivory staminate flowers (Lovell, 1902). Pistillate flowers, which are usually born distally, are not conspicuously colored (Lovell, 1902); but, the blossoms do produce nectar (Sapkota et al., 2010).

In chestnut trees, insects mostly perform a passive function by dislodging the pollen from the anthers (male flowers) (Hardin et al., 2001). Manos et al. (2001) suggested the possibility that various features of castaneoid flowers, such as insect pollination, are preserved plesiomorphies (Manos et al., 2001). The fact that *Castanea* uses a combination of anemophilous and entomophilous dispersal strategies may be a result of an evolutionary

intermediacy between the derived anemophily trait of other Fagaceae and ancestral entomophily (Hardin et al., 2001).

Manos et al. (2001) concluded that within Fagaceae, wind pollination has been derived twice and hypogeous germination once based on examinations of phylogenetic relationships using a combination of nuclear DNA and chloroplast analyses (Manos et al., 2001).

Castanea has evolved pollen selection mechanisms that reduce inbreeding depression and promote outcrossing. One of these mechanisms is self-incompatibility. Another is inbreeding avoidance, in which pollen from more distant and unrelated parents are more likely to father seeds. Hasegawa et al. (2009) demonstrated that rates of pollen tube growth and pollen germination were lower for pollen grains from related individuals than for those from non-related ones (Hasegawa et al., 2009).

APPENDIX B

ORCHARD CARE AND MAINTENANCE

When initially establishing an orchard, weed control is critical as vegetation competes with trees for soil nutrients, space, and water resources (Payne et al., 1982). Indeed, some authorities have argued that controlling competing vegetation is the most important cultural practice during the establishment of trees (Vossen, 2000). Herbicides, mowing, and pulling weeds by hand can be combined to control grass and other competitors (Payne et al., 1982). About 1 to 2 meters around the tree should be competition-free. As an alternative to herbicides, synthetic fabric mulches can also be used. Close mowing prior to harvesting will ensure a lower percentage of nuts lost in the vegetation (Vossen, 2000).

Growers usually decide before site preparation how they are going to start off their cultivars. A chestnut orchard can be initiated by planting seedlings and field grafting one to two years later or by planting already grafted trees. Although the latter method is the easiest, it is at a higher risk of transplant shock and graft failure. Grafted trees start to bear fruit two to three years after the cambium layers become integrated; and most fruit orchardists grow reliable cultivars (Hunt et al., 1998). However, in an experimental planting in Byron, Georgia, 10-yr old seedlings were able to produce a reported 4,400 kg/ha (2 tons/acre) from 70 seedlings (Payne et al., 1982). Also, a study by Martin et al (2009) suggested that geography may affect the juvenile period of a seedling. For example, in Andalusia (southern Spain), the juvenile period is 3-4 years, which is much shorter than the usual 15 years required for fruit maturation in other localities (Martin et al., 2009). Such productivity has a great potential for generating profits. Therefore, it may be worthwhile to consider planting seedlings as well as standard recommended cultivars.

There still remains the long-standing dilemma of whether to grow seedlings or grafted trees which has persisted throughout chestnut growing, and it is no less relevant here. The advantages of seedlings are that they are easily and less expensively propagated. The benefits of named varieties are that they are uniform and have a shortened time period between propagation and harvest; but, they are more expensive, difficult to propagate, and delayed graft incompatibility is a frequent concern with them (Moore, 1948).

Whether the grower is planting seedlings or grafts, young trees need to be watered and protected from weeds more thoroughly in the early years. One can obtain grafted trees in containers or as a bareroot tree, the latter of which is more affordable but has more trouble with transplant shock (Hunt et al., 1998). Seedling chestnut trees are easily procured and inexpensively bought compared to grafted ones. Although bareroot trees have a high survival average, their growth will progress at a reduced rate. One may purchase more chestnut seedlings and a few cultivars at first, then obtain scion wood and graft the seedlings one or two years later. However, this will result in

slightly delayed profits with the additional expenditure of grafting one's own trees or hiring a grafter (Hunt et al., 1998).

If an orchard manager decides to use container-grown trees, they can be generated by stratifying fresh nuts in moistened sand or storing in resealable plastic bags with damp sphagnum moss in a refrigerator set to -1.1°C-4.4°C or a chilled room for 60 to 90 days. The bags should be examined often and spoiled nuts removed. The taproot will have sprouted when the time comes to plant, so extraordinary precaution must be taken to avoid breaking the radicle. If the nuts are planted in the field, they should be planted after the danger of frost passed (Hunt et al., 1998).

According to the Farmers' Almanac, the average last spring frost occurs in Chattanooga on April 1 and the average first fall frost around November 4. Generally three categories of freezes are recognized: light, moderate, and severe. A light freeze is between -1.6 and 0ºC and kills mostly delicate plants. A moderate freeze is between -3.8 and -2.2ºC and damages most vegetation, particularly fruit blossoms. A severe freeze is -4.4ºC and colder and is destructive to most plants (FarmersAlmanac, 2007).

Site and water requirements

In regional studies conducted to determine locational preferences for European chestnut (*Castanea sativa* Mill.) in northwestern Spain, it was found that European chestnuts thrived more on former agricultural lands than they did on former forest land. High concentrations of K, P and Ca, high summer precipitation, low elevation, and more frost free days resulted in greater height and diameter growth, whereas high foliar N and Al levels had the lowest growth rates. Overall, however, nutrition was less critical for growth on former agricultural lands than on forested lands, which may indicate that areas that were previously cultivated for agricultural uses generally have better soil nutrition. Thus, if growing an orchard on a formerly forested site that has high organic matter and poorer nutrition, more intensive site preparation and fertilizer applications may be necessary (Álvarez-Álvarez et al., 2010).

Fortunately, chestnut trees can thrive in a variety of sites, soils, and microclimates, which gives them an advantage over other crops that are unsuitable. The main sites which chestnut trees do not tolerate are areas with standing water and/or highly alkaline soils (Vossen, 2000).

Although most Chinese chestnuts can handle -20ºF temperatures, one should aim for sites that are not frost-pockets. In general, the sites that are suitable for peaches are capable of meeting the requirements for Chinese

chestnuts: areas in which summit and shoulder slopes permit adequate air flow and reduce the risk of injury to swelling buds in the spring (Hunt et al., 1998).

Chestnut tree care

Hedge-row planting systems, where trees are more closely spaced within rows than between rows, are more efficient with regards to irrigation and floor management (Vossen, 2000). One hedge-row method, developed by Dr. Hitoshi Araki, involves a nut-maximizing hedgerow system that is presently used in Australia and New Zealand which consists of trees planted with a 4 meter distance of each other in rows spaced 8 meters apart (Hunt et al., 1998). This system developed out of research in Japan which suggests that chestnut trees require high light intensity, as there is a direct correlation between the number of flowers per cubic foot of canopy and radiation intensity. Large fruit size is facilitated by high sunlight intensity which promotes shoot-growth. This scheme maximizes the penetration of sunlight into the crown. A stipulation of this system is to limit by pruning the height of trees to 3.65 to 4 meters and restrain the length from the center of the tree to the edge of the crown (Hunt et al., 1998).

Soil management practices

There are a variety of soil management practices for chestnut orchards. Although chestnut trees are rather drought tolerant, water deprivation leads to smaller nut size and lower yields, and thus, lower profits. To combat small nut size resulting from periods of drought, drip-irrigation systems can be applied relative to the amount of rainfall (Vossen, 2000). However, there are a variety of ways of implementing an irrigation system; but some studies suggest that certain methods work better for chestnut orchards than others.

For instance, in a report published by Martins et al (2011) on the effects of different soil management regiments in chestnut orchards, the productivity, pathology, and physiologic responses of chestnut trees were observed with regards to tree, water, and soil interactions. The study compared conventional tillage systems (CT), no tillage with permanent spontaneous vegetation cover (NV), no tillage with permanent rain-fed seeded pasture cover (NP), and no tillage but with irrigation (NIP). The responses measured were fruit and edible mushroom production (Martins et al., 2011).

Conventional tillage was associated with increased soil compaction, erosion, loss of organic matter, decline of soil nutrients, tree root damage, and increased risk of ink disease. Other studies also suggest that, during drought

conditions, conventional tillage is more injurious to chestnut orchards, but, when tillage is used in conjunction with natural vegetation cover, fruit productivity and soil quality increases in Mediterranean agro-ecosystems. In the soil management study, maintaining soil cover decreased erosion, increased biological species richness, and increased the overall efficiency of the system. These silvo-arable and silvo-pastoral arrangements allow for both market and nonmarket benefits, the latter being soil conservation, biodiversity, and improved air and water quality. Maintaining sustainable high quality soil requires microbial diversity and beneficial saprotrophic and mycorrhizal fungi. Agro-ecosystems can thus acquire plant nutrition, drought tolerance, and security against root diseases such as *Phytophthora*. Hence, sorocarps of ectomycorhizal fungi are used as indicators of soil quality (Martins et al., 2011).

In the previously mentioned comparative study between conventional and no-tillage schemes, they reported no-till systems as being generally more conducive to promoting fungal communities. Such management practices enhanced tree productivity in chestnut orchards as well as producing prized edible sorocarps such as *Boletus edulis, B. aestivalis*, *B. aereus,* and *B. pinophilus*. The potential for producing other economically valuable ectomycorrhizal symbionts of chestnut trees may serve as a motivation for chestnut producers to transition from conventional cropping systems to no-tillage systems in a multifunctional orchard context. A wide range of uses in a chestnut orchard may be exploited with proper management, including nuts, pasture, and edible mushrooms. However, in order to formulate such schemes, a collaborative effort between researchers and growers should develop guidelines for sustainable soil management (Martins et al., 2011).

The system which produced the highest productivity of edible mushrooms was no tillage but with irrigation; and the arrangement which created the lowest total sorocarp biomass was the conventional tillage treatment. Thus, research suggests that productivity of commercially marketable mushrooms can be improved with irrigation. There was also higher species richness in the NIP and NV treatments than the CT treatments (Martins et al 2010).

The NP and the NIP treatments showed the highest annual net income for nuts and edible mushrooms, NIP being the highest. However, due to the high economic inputs and possible higher environmental costs, the no-tillage system with natural vegetative cover may be more advantageous than irrigation treatments in areas where water resources are scarce. The no-tillage strategy also may increase soil organic content, which is critical for a sustainable agroforestry system. Summarily, a multifaceted operation of chestnut orchards will likely enhance economic diversification, biodiversity, nut production, and more environmentally sustainable plantations (Martins et al., 2011).

APPENDIX C

STORING CHESTNUTS

Proper storage is very critical in the post-harvest process. Since chestnuts are living tissue, the only way to slow down their respiration is to reduce the temperature (Mencarelli, 2003). During storing, the chemical composition of the fruit is changed, fructose and other sugars increase and starch is decreased (Jermini et al., 2006).

Ancient methods of storing chestnuts consisted of laying the nuts out, still in their husks, on mounds and covering them with leaves, sands or other earth and moistening them periodically. The nuts would then ferment, prolonging their storability. A wood hammer (called a *picot* in Italian) was used to separate the husks from the nuts. The Cuneo area in particular employed this method, or the nuts were put into a sac made of animal skin or other material and beaten against a hard surface, such as a tree trunk (Bounous, 1999). Sand storage, a traditional chestnut storage method, is still used in China today. It has a high percentage (30-40%) of chestnut loss, though (Mencarelli, 2003).

Another traditional approach involves soaking the nuts in water from 4 to 10 days at room temperature. This curing procedure eliminates defective nuts that float. The next step is to disperse the nuts in layers and agitate them regularly until their surfaces are dry or use some type of ventilation (Bounous, 1999). In Italy, the most common post-harvest treatment is water curing. The reasons for water curing are to reduce the development of fungi during storage and to kill worms in the chestnut. In this method, lactic acid and alcohols are partially fermented, which lowers the pH and diffuses phenols from the episperm into the nut kernel. In the past, this procedure took up to nine days but has now been reduced to about three days (Mencarelli, 2003).

The Agriculture and Consumer Protection Department of the Food and Agriculture Organization recommends the water in the curing treatment to be at 15°C and the chestnuts submerged for 3 to 7 days. They indicate that fermentation is indicated by bubbling in the water. By the end of the water immersion, the pH will have reduced by nearly 1 point. The chestnuts are then spread out in a layer of 5-10 cm thick and an air stream flows over them to dry out external moisture. They are rotated every day until transport (Mencarelli, 2003).

Chestnuts can also be immersed in hot water baths (50ºC) and shocked in cold water. 50ºC is the temperature at which chestnuts' proteins are not denatured and the allotted time guarantees that insect larvae and eggs are killed. If dried chestnuts are the intended product, then drying proceeds. The kernel can also be sterilized via methyl bromide fumigation. Cold storage can be used in conjunction with curing and Cold Atmospheric Storage (CAS) in a facility with adequate ventilation and 90-95% humidity at 0-2ºC for 3-4 weeks. Cold storage is similar to CAS but the storage is longer (3-4 months) in a cold room with oxygen levels at 5% and CO_2 levels at 5-10%

(Bounous, 1999). Germination studies suggest that optimal storage temperatures for chestnuts are between -6º and 2ºC, as this temperature range results in a higher percentage of germinated nuts after storage (Payne et al., 1982). However, Jermini et al determined that below 0°C was the optimal storage temperature to prevent the development of fungi (Jermini et al., 2006). Alternatively, chestnuts destined for candying and *marrons glacés* can be frozen whole or peeled for 6-12 months in freezers at temperatures of -18ºC to -20ºC (Bounous, 1999). It is also important to note that different treatments before storage can also have effects on the nuts' quality.

This was demonstrated by Mujić et al (2008), in which the influence of different storage techniques on starch digestibility and microorganism presence was evaluated. The least mold contamination was on the nuts treated with a sage solution and stored at 2°C. Samples with the least bacteria were those treated with an antimicrobial solution comprised of sage and rosemary. The optimum storage preservation method which resulted in the least contamination was storage at -18°C and a sage and rosemary post-harvest treatment. At 20°C, chestnuts lost almost all their moisture within 60 days, which reduced their resistant starch content owing to the necessity of water for the re-crystallization of amylase. Frozen chestnuts had no alteration in their resistant starch content and no mold or yeast contamination (Mujić et al., 2008).

In similar study conducted by Jermini et al (2006) to evaluate the effects of different treatments on chestnuts' storability, 8 different fruit parameters were analyzed including nut mass, glucose, water content, fructose, sucrose and the presence of insects and molds. The response variable included peel-ability, sweetness, aroma, texture and acceptance from taste tests. The results showed that the longer the storage, the more weight and water content decreased. The longer chestnuts were submerged in water, the higher the water absorption, which is probably due to the reduction of fruit transpiration in anaerobic conditions (Jermini et al., 2006).

For all lengths of storage time, a decrease in starch content was correlated with an increase in sucrose content. The soaking treatment always showed a significantly higher proportion of diseased and moldy nuts. Jermini et al (2006) determined that below 0°C was the optimal storage temperature to prevent the development of fungi. The most common storage mold species detected were *Ciboria batshiana* (Zopf), *Penicillium* spp. (Link), *Mucor hiemalis* (Wehmer), and, in a few samples, *Amphiporthe castanea* (Tul. & C. Tul.) and *Trichoderma* spp. (Person) (Jermini et al., 2006).

The proportion of nuts that had living larvae pests was significantly higher in the soaking treatment than in the warm and cold bath treatments. Of all the treatments, the warm bath killed almost all insect larvae most

effectively. In the sensory evaluation, the warm-bath treated nuts scored the highest. In peel-ability tests, the soaked nuts scored the worst, but cold and warm bath treated nuts scored equally well. The warm bath treated nuts were rated as having the sweetest flavor, and the cold bath nuts were rated as being the least sweet (Jermini et al., 2006). It seems, then, that warm bath treated nuts have the most positive aspects.

There are practical benefits of cold bath treatments, however. They require less energy and less expensive equipment and increase the nut weight; but, there are a number of disadvantages. Cold baths require more space and they decrease the sweetness of the nut. The nuts cannot be moved for 6 to 10 days. The nuts become duller in appearance, and finally, cold baths do not even completely eliminate *C. elephas* larvae. Although warm bath treatments are more intensive procedures, they are faster operations; they increase sweet flavors, and nearly guarantee the complete elimination of insect larvae. Therefore, warm bath treatments are probably the most expedient way to pre-treat chestnuts for storage (Jermini et al., 2006).

During the handling, storage, and processing of chestnuts, controlling browning is one of the most important challenges. Browning, which is partially triggered by polyphenol oxidase (PPO) enzyme, negatively affects chestnuts' flavor and appearance, and thus, their marketability. PPO activity is influenced not only by cultivar and the maturity of the tree, but also by postharvest handling. What the optimum conditions are for enzyme activity in chestnut PPO content is an important question. Xu (2005) assessed the function of PPO in the storage and processing of *C. henryi* chestnuts and found this enzyme to be heat labile. This attribute might be useful in checking enzymatic browning during processing and storage of chestnuts (Xu, 2005).

Sorting

Modern methods of sorting include rotating drums which separate the chestnuts based on size. Insect or fungus-damaged defective nuts are usually manually sorted on conveyor belts. Dirt and powdery mildews can be removed by a series of brushers, which also polishes the chestnuts. Fresh chestnuts are packed in plastic mesh sacks and are mostly consumed in the fall season (Bounous, 1999).

Drying

Traditional methods of drying consist of a slatted floor with a pile of chestnuts 0.6 m to 0.9 meters thick on top with smoke rising through from a fire in the basement (Smith, 1950). Drying sheds employ two layers: the upper

layer consists of wood or metal grating with chestnuts on them, while below a constant heat is supplied from burning wood or chestnut peels. During this process the chestnuts are turned and their internal temperature is monitored. The operation takes about 30 days. Modern electrical ovens that disperse heat more evenly and take much less time can also be used (Bounous, 1999).

APPENDIX D

NUTRITIONAL CONTENT OF CHESTNUTS

Chestnuts are dense in energy, primarily in the form of carbohydrates; but they also supply vitamins and minerals (Smith, 1950). Commercially, chestnuts are considered "nuts"; however, the pulp is 46-50% water (approximately) and is only 5% lipid. Thus, it should be treated more as fresh produce than a traditional, oily nut. They are desirable for their nutritional qualities as they provide minerals such as sulfur, calcium, phosphorous, complex carbohydrates, some high quality protein (about 1.6g/100g), vitamins C and B, and are besides low in fat (Bounous, 1999). However, not all chestnuts are alike. There are slight nutritional differences between species (Senter et al., 1994).

Assays conducted on the nutrient content of chestnuts show that the primary saturated fatty acid in Chinese and American chestnuts is palmitic, while the predominant unsaturated fatty acid in American and Chinese chestnuts is oleic. Linoleic acid is the major unsaturated fatty acid in European chestnuts. Sucrose quantities are not significantly different by species. Fructose and glucose are present in all species but in low quantities. As the fat content in all chestnuts is mostly unsaturated, they have a high nutritional quality (Senter et al., 1994).

As chestnuts are stored, their starch content converts to sugars. The disaccharide content is the most important property in consumers' taste preference. Resistant starch (starch which escapes digestion in the small intestine) comprises 42.2-43.2% of total starch in *marroni* cultivars from the Latium region in Italy, and rapidly digestible starch is 15.8%. High concentrations of soluble carbohydrates make long term storage of chestnuts problematic, though. An unsuitable storage environment can result in uncontrolled enzymatic reactions which may affect taste, texture, color and nutritive values (Mujic et al., 2008).

VITA

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