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Growth and Cropping of 'AU-Super' or 'Eaton' Chestnut Trees with 'Little Giant' Interstem on AU-Cropper Seedling Rootstock

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Abstract

Chestnut (*Castanea* sp.) scion cultivars, 'AU-Super' and 'Eaton', with or without 'Little Giant' interstem on AU-Cropper seedling rootstock, were grafted in 2005 and planted in 2006 near New Franklin, MO to evaluate tree survival, vegetative growth, and nut yield for eight growing seasons. Tree survival in 2013 was 100% for each cultivar/interstem combination, except for 'AU Super' with 'Little Giant' interstem which had a 22% loss due to delayed graft union failure. 'Little Giant' interstem reduced tree height and trunk cross-sectional area (TCA) of 'AU-Super' trees by 0.7 m and 57 cm^2, respectively, but did not affect height or TCA of 'Eaton' trees. Trees of both cultivars with or without the interstem exceeded their allotted 4 m in-row spacing in 2012. Trees of three scion/interstem combinations produced a few chestnuts in 2008, whereas 'Eaton'/'Little Giant' interstem trees began cropping in 2009. The largest annual increase in nut yield per tree for all scion/interstem combinations was in 2012. Average nut weights for all trees were greatest in 2010, but were reduced thereafter. By 2013, 'AU-Super' trees had greater cumulative yield than 'Eaton' trees, but it was not affected by the interstem for either scion cultivar. Cumulative yield efficiency was similar for cultivars with or without interstems. Thus, 'Little Giant' interstem grafted to scion cultivar 'AU-Super' and 'AU-Cropper' rootstock had a slight dwarfing influence on trees and did not adversely affect cumulative nut yield from 2008 to 2013. However, as cropping generally increased, average nut weights for all cultivar/interstem combinations were substantially reduced in the sixth growing season and remained low through 2013.

In 2011, worldwide chestnut production was 2.02 Mt, while that in the United States was ≈ 680 t (FAO, 2013; Warmund, 2011). Because large, fresh chestnuts command a high price in the marketplace, nut size influences cultivar selection (Warmund, 2011). Cultivar recommendations vary widely by region, but Chinese chestnut (*Castanea mollissima)* cultivars are often grown in the eastern United States, where low temperature injury and chestnut blight (*Cryphonectria parasitica*) are problematic on other chestnut species (Metaxas, 2013; Vossen, 2000; Warmund et al., 2010). In this area, 'Eaton' [*C. mollissima* x (*C. crenata* x *C. dentata*)] is a commonly grown cultivar. Although results from long-term cultivar trials have not been published, Anagnostakis (2007) reported that 'Eaton' produces nuts averaging 14 g, whereas some Chinese chestnut cultivars, including 'AU-Super', produce large chestnuts averaging up to 25 g/nut.

One of the major limitations to expanded chestnut production is the use of large trees planted at wide spacings with low nut yields. Common tree densities for European (*C. sativa*) and Japanese (*C. crenata*) chestnut are 100 and 285 trees/ha, respectively (Bounous and Marinoni, 2005). In North America, the recommended planting density for Chinese (*C. mollissima*) and Japanese x European hybrids is 123 trees/ha (Hunt et al., 2012).

One approach to enhancing chestnut yield efficiency is to plant naturally dwarf trees at a high density. Chinese chestnut cultivars with a dwarf tree stature, including 'Yanshanduanzhi', 'Beiyu No. 2', 'Yanchangli', 'Yimengduanzhi', and 'Zunyou No. 5' have

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been selected in China, but this germplasm is not available in North America (Qin et al., 2005). Alternatively, grafting a chestnut cultivar onto a dwarfing rootstock or grafting a scion onto an interstem from a dwarf cultivar might be used to reduce tree size and increase yield efficiency. However, little rootstock research has been conducted in North America and clonal rootstocks are unavailable from commercial nurseries. In the central region of the United States, 'AU-Cropper' is often used as a seedling rootstock because it is compatible with a wide range of Chinese cultivars and their interspecific hybrids (Harris et al., 1980; Warmund, 2011).

'Little Giant' (complex hybrid of *C. mollissima* x *C. seguinii)* is a release from the Connecticut Agricultural Experiment Station and trees are relatively small compared with other Chinese chestnut trees at maturity (S.L. Anagnostakis, pers. comm.). Metazas (2013) reported that 'Little Giant' on seedling *C. mollissima* rootstock produced small nut size (< 7 g/nut). While 'Little Giant' may be unsuitable as a scion cultivar, it may be useful as an interstem to induce dwarfing. Therefore, this study was conducted to evaluate tree growth and nut production of 'AU-Super' and 'Eaton' cultivars with or without 'Little Giant' as an interstem on AU-Cropper seedling rootstock over an eight-year period. Nut number, yield, and average nut weight were also analyzed to further examine annual bearing characteristics of these trees.

Materials and Methods

Nuts were harvested in September 2003 from 'AU-Cropper' Chinese chestnut trees planted in a repository in 1996 at the Horticulture and Agroforestry Research Center (HARC), New Franklin, Missouri to produce seedling rootstocks. Immediately after harvest, chestnuts were sealed in polyethylene bags and placed in cold storage at 5ºC. On 15 Mar. 2004, these chestnuts were sown in 35 x 35 x 13-cm (depth) flats using a 8 pine bark: 4 perlite: 2 sphagnum peat moss: 1 vermiculite: 1 sand (v/v) medium amended with 2 kg Osmocote 13N-5.7P-10.8K, 1.5 kg Nitroform Blue Chip 38N-0P-0K (AgrEvo, Wilmington, DE), and 1.7 kg Micromax micronutrients (Scotts Co., Marysville, OH) per m^3. Seedlings were transplanted into 11.4-liter polyethylene containers on 1 May 2004 using the medium previously described. Potted seedlings were grown outdoors in a nursery area at HARC under 55% shade cloth (DeWitt Group, Sikeston, MO) with supplemental irrigation as needed. From 25 Nov. 2004 to 1 Apr. 2005, rootstock seedlings were covered with a foam blanket and white polyethylene sheeting for winter protection.

'Little Giant' interstem wood was collected from mature trees at the Connecticut Agricultural Research Station, New Haven, CT on 1 Mar. 2005, sealed in polyethylene bags, and shipped by overnight mail to the University of Missouri where it was held at 5°C until grafting. 'AU-Super' and 'Eaton' scion wood was collected at HARC on 1 Mar. and then cold-stored until grafting.

On 2 May 2005, 12 trees of each cultivar/interstem/rootstock combination were grafted. Using the whip-and-tongue technique, either an 'AU-Super' or 'Eaton' scion piece with two buds was grafted onto a 10 cm-long 'Little Giant' interstem piece. Because the previous season's growth of 'Little Giant' trees was < 15 cm-long at the time of collection, interstem pieces used for grafting were relatively short. Immediately after the first graft was completed, this scion/interstem piece was grafted onto an AU-Cropper rootstock at 8 cm from the potting medium surface using the same procedure. To propagate trees without an interstem, AU-Cropper rootstocks were whip-and-tongue grafted at 8 cm above the potting medium surface with two-bud scions for each scion cultivar. Grafted tissue was tied with a rubber budding band, sealed with wrapping film (Parafilm M, Bemis North America, Oshkosh, WI) covered with aluminum foil, and placed in a greenhouse at 27°C day/21°C night on a 12 h cycle for 3 weeks with natural lighting. Aluminum foil and budding bands were removed and placed in a field nursery under 55% shade cloth (DeWitt Group, Sikeston, MO) with supplemental irrigation as needed. On 25 Nov. 2005, potted trees were covered with a polyethylene foam blanket for winter protection and uncovered on 1 Apr. 2006.

On 13 Apr. 2006, 9 trees (replications) of each cultivar with or without interstem were planted in a deep, upland Menfro silt loam soil (fine-silty, mixed, superactive, mesic typic hapludalfs) at HARC with the lowest graft union 10 cm above the soil surface. Trees were spaced 4 m within the row and 8 m between rows and were arranged in a randomized complete block design. Trees were maintained in their natural form with little pruning. Drip-irrigation scheduling and pest and fertility management followed local recommendations (Hunt et al., 2012). Fertilizer (34N-0P-0K) was applied underneath the trees to the dripline of the canopy at 67 g/tree on 20 Apr. 2006. Because an unprecedented freeze event damaged catkins and new foliage in early April 2007, fertilizer application (67 g/tree) was delayed until 28 June. Fertilizer (34N-0P-0K) was applied on 27 Mar. and 1 June at 82, 250, and 400 g/tree in 2008, 2009, and 2010, respectively. In subsequent years, fertilizer (34N-0P-0K) was applied at 520 g/tree on 27 Mar. and June 1.

Trunk circumference at 15 cm above the lower graft union was measured on 28 Oct. 2013 and trunk cross-sectional area (TCA) was calculated. Tree survival and height were recorded on 25 Nov. 2013. The number of primary nuts (i.e., produced from burs set in June) and the total weight of chestnuts harvested per tree were recorded annually when chestnut production began in 2008 through 2013. Average nut weights were calculated from these data. Because secondary nuts (set in early August) were unmarketable (< 3 g/nut), they were excluded from the study. Chestnuts were harvested every other day over a 20-day period after they naturally dropped to the ground. Cumulative yield and cumulative yield efficiency (six year cumulative yield divided by TCA in 2013) were calculated. Data for TCA, tree height, cumulative yield per tree, cumulative yield efficiency and average nut weight were subjected to analysis of variance using the MIXED procedure of SAS (Version 9.2; SAS Institute, Cary, N.C.). These variables were checked for normality using the residuals from ANOVA using PROC UNIVARIATE and were found to be normally distributed. Means were separated by Fisher's protected least significant difference (LSD) test, $P \leq 0.05$. Nut number, yield, and average nut weight for each year was analyzed as a split-plot design with repeated measures to evaluate annual changes in these variables using the MIXED procedure of SAS. Since nut number data were not normally distributed, repeated measures analyses were performed on ranked data as described by Conover and Iman (1981). Means were separated by Fisher's protected least significant difference (LSD) test, $P \leq 0.05$.

Results

After eight growing seasons in the field, tree survival was 100% for each cultivar/interstem/rootstock combination, except for 'AU Super' with 'Little Giant' interstem. Two of nine trees of this cultivar/interstem combination exhibited symptoms of delayed graft union failure and died in fall 2006. By 2013, 'AU-Super' tree growth was affected by the interstem (Fig. 1). 'AU-Super'/'Little Giant'/AU-Cropper trees had smaller TCA and were shorter than 'AU-Super' trees without the interstem (Table 1). 'AU-Super' trees with or without interstem also had greater TCA than that of 'Eaton' trees. 'Little Giant' interstem did not affect TCA or height of 'Eaton' trees.

Cumulative yield per tree from 2008 through 2013 was affected by cultivar, but not by interstem (Table 1). 'AU-Super' trees had greater cumulative yield than those of 'Eaton'. However, cumulative yield efficiency was similar for all scion/interstem combinations. Average nut weight (from

Table 1: Trunk cross sectional area, tree height, cumulative yield, cumulative yield efficiency, and average nut weight of 'AU-Super' and 'Eaton' Chinese chestnut on AU-Cropper seedling rootstock with or without 'Little Giant' interstem.[z]

Cultivar	Interstem	2013 TCA (cm^2)	2013 Tree height (m)	2008-2013 Cumulative yield (kg/tree)	2008-2013 Cumulative yield efficiency ($kg\cdot cm^{-2}$)	2008-2013 Average nut wt. (g)
AU-Super	no	224 a	5.7 a	38.9 a	0.17	14.4 a
AU-Super	yes	167 b	5.0 b	31.4 a	0.19	12.6 b
Eaton	no	112 c	5.1 b	19.3 b	0.17	13.8 ab
Eaton	yes	107 c	4.9 b	18.6 b	0.17	12.6 b

[z] Values represent means of nine single-tree replications of each cultivar/interstem/rootstock combination. Means within columns followed by different letters are significantly different (Fisher's protected least significant difference test, $P \leq 0.05$).

2008 to 2013) was greatest for 'AU-Super'/AU-Cropper trees and smallest for trees with interstems (Table 1).

In 2008, none of the 'Eaton'/'Little Giant' interstem trees produced chestnuts, whereas trees of the other three scion/interstem combinations produced ≤ 45 nuts/tree (data not shown). When annual production was analyzed from 2008 to 2013, 'Little Giant' interstem did not affect nut number or yield (Table 2). Nut numbers per tree for each scion cultivar increased annually, with the maximum number of chestnuts harvested in 2013. However, 'AU-Super' trees produced more nuts than 'Eaton' trees annually, except in 2010. Nut yields for both scion cultivars increased numerically with tree age, with the largest annual increase in yield per tree in 2012. Additionally, annual nut production for 'AU-Super' trees was greater than that of 'Eaton' trees in 2011, 2012, and 2013.

Fig 1: Trunk of a chestnut tree with 'Little Giant' interstem (between arrows) that was grafted onto 'AU-Super' scion and AU Cropper seedling rootstock in 2005.

Average nut weights were generally the greatest for each scion/interstem combination in 2010 (Table 3). Average nut weight for 'AU-Super'/'Little Giant'/AU-Cropper trees was greater than that for 'Eaton' trees with or without an interstem in 2010. However, average nut weights in 2011 were significantly lower than those in 2010 for each scion/interstem combination and generally remained low in 2012. By 2013, chestnuts harvested from all 'AU-Super' trees had lower average nut weights (≤ 8.3 g/nut), than those from 'Eaton' trees with or without an interstem (12.7 g and 12.4 g, respectively).

Discussion

Although propagation efficiency was not a specific objective of this study, grafting all three components of the chestnut tree on the same date in late spring using the whip-and-tongue technique was an effective method for producing trees with interstems. Fruit trees

Table 2. Annual nut number and yield of 'AU-Super' and 'Eaton' Chinese chestnut trees on AU-Cropper seedling rootstock.

Cultivar	Year	Nut no./tree[z]	Yield/tree (g)
AU-Super	2008	40 h	186 gh
AU-Super	2009	62 g	1088 fgh
AU-Super	2010	96 f	2048 f
AU-Super	2011	401 d	3741 e
AU-Super	2012	1188 b	15503 b
AU-Super	2013	2050 a	16610 a
Eaton	2008	2 i	8 h
Eaton	2009	31 h	252 gh
Eaton	2010	68 fg	1268 fg
Eaton	2011	124 e	1826 f
Eaton	2012	08 d	6438 d
Eaton	2013	729 c	9164 c

[z] Values represent means of 18 single-tree replications of each cultivar/rootstock combination before data transformation for statistical analyses. Means within columns followed by different letters are significantly different (Fisher's protected least significant difference test, $P \leq 0.05$).

with interstems are often produced in the nursery over multiple growing seasons using rooted layers grown for one year (Hartmann et al., 2010). In one method, rooted layers are planted in nursery rows in early spring and are then chip-budded with the interstem that fall. A year later, a scion cultivar is budded to the interstem/rootstock in the field. Alternatively, the rootstock may be grown, dug from the field, and one or two bench grafts are made and stored for callusing indoors before lining out grafted fruit trees in the field to grow for one or two seasons. Thus, the spring grafting technique used in this study eliminated the need to cold-store grafts for union callusing or to propagate trees with interstems for multiple growing seasons.

This study also demonstrated 'AU-Super' without 'Little Giant' interstem is a more vigorous scion cultivar than 'Eaton' when grafted onto AU-Cropper rootstock. Additionally, a 10 cm-long interstem piece reduced TCA and height of chestnut trees with the scion cultivar 'AU-Super', but not 'Eaton' (Table 1). However, the use of a longer interstem piece might dwarf trees with 'Eaton' as a scion cultivar and further reduce the height of 'AU-Super' trees. Carlson and Oh (1975) reported that the length of the dwarfing interstem determines apple tree size, with a 30 cm-long interstem producing a shorter tree than that with a 10 cm-long interstem.

By harvest 2012, trees filled their in-row spacing. In spite of the reduced tree size of 'Eaton' trees and 'AU-Super' with an interstem, a 4 m spacing within the row resulted in branch overlap among trees well before they reached maturity with AU-Cropper rootstock. In spite of the branch overlap in 2012, nut yield increased in 2013, presumably from burs borne on sun-exposed terminal growth of the outer portion of the tree canopy where flowers are typically produced (Miller, 2003). Current recommendations for grafted Chinese chestnut trees grown in Missouri are 9 m x 9 m for an initial spacing, with half the trees removed 15 years after planting (Hunt et al., 2012). At the spacing used in this study, alternate tree removal would be needed in the 6th year after planting. To maintain the 4 m in-row tree spacing used in this study and prolong the life the orchard, a less vigorous rootstock than AU-Cropper or a more dwarfing interstem is needed to increase tree density and yield efficiency. Additionally, cultivars with a more upright or compact growth habit than those used in the present study might be used to delay the time before the in-row space is filled.

Interstems did not greatly affect precocity of the trees (Table 2). While 'Eaton'/'Little Giant'/AU-Cropper trees failed to flower and bear nuts in 2008, other trees produced few nuts that year. Also, for each scion, the interstem did not affect annual nut number or annual yield per tree (Table 2).

According to Hunt et al. (2012), a commercial Chinese chestnut production level (≥ 834 $kg \cdot ha^{-1}$) is expected six to nine years after grafting when planting at conventional tree densities (123 trees/ha). Using the tree density in the present study (316 trees/ha), 'AU-Super' trees would exceed this commercial production level in 2011 (6 years after grafting), whereas 'Eaton' trees would surpass this yield in 2012. In contrast, using the yield/tree values obtained in this study for 123 trees/ha, production of ≥ 834 $kg \cdot ha^{-1}$ would occur for 'AU-Super' and 'Eaton' trees in 2012 and 2013 (7 and 8 years after grafting), respectively. Another benchmark for Chinese chestnut production is attaining ≥ 2242 $kg \cdot ha^{-1}$ by tree age 12 to 15. At the density used in this study, 'AU-Super' trees exceeded this production in 2012 at seven years after grafting, with numerically greater yields in 2013. 'Eaton' trees did not surpass > 2242 $kg \cdot ha^{-1}$ until 2013.

While high yields were recorded for these trees, average nut weight for all scion/interstem combinations was greatly reduced from 2010 to 2011 (Table 3). For 'AU-Super' trees, the reduced average nut weight occurred after there were >96 nuts/tree or > 2.0 kg/tree yield (Tables 2, 3). For 'Eaton' trees, the large drop in average nut weight occurred after trees had > 68 nuts/tree or >1.3 kg/tree yield. For the fresh market, large nut size is preferred, but standards

Table 3. Average nut weight of 'AU-Super' and 'Eaton' Chinese chestnut trees on AU-Cropper seedling rootstock with or without 'Little Giant' interstem.[z]

Cultivar	Interstem	Year	Ave. nut wt. (g)
AU-Super	no	2008	21.5 ab
AU-Super	no	2009	18.2 cd
AU-Super	no	2010	20.6 abc
AU-Super	no	2011	9.2 h
AU-Super	no	2012	9.8 gh
AU-Super	no	2013	7.9 i
AU-Super	yes	2008	9.9 gh
AU-Super	yes	2009	16.8 de
AU-Super	yes	2010	23.0 a
AU-Super	yes	2011	9.6 gh
AU-Super	yes	2012	9.6 gh
AU-Super	yes	2013	8.3 i
Eaton	no	2008	7.5i
Eaton	no	2009	16.3 de
Eaton	no	2010	19.1 bcd
Eaton	no	2011	14.7 ef
Eaton	no	2012	12.7 fg
Eaton	no	2013	12.4 fgh
Eaton	yes	2008	---[y]
Eaton	yes	2009	16.2 de
Eaton	yes	2010	18.1 bcd
Eaton	yes	2011	14.7 ef
Eaton	yes	2012	12.6 fg
Eaton	yes	2013	12.7 fg

[z] Values represent means of nine single-tree replications of each cultivar/interstem/rootstock combination. Means within columns followed by different letters are significantly different (Fisher's protected least significant difference test, $P \leq 0.05$).

[y] --- No nuts harvested from trees.

for nut size have not been adopted by the United States Department of Agriculture (Hochmuth et al., 2013). Various size classifications have been proposed using nut diameter, nut weight, or number of nuts per unit weight (Chong, 2013; Hochmuth et al., 2013; Warmund et al., 2010). In one grading scheme, chestnut grades ranged from grade A (11.6 to 12.6 g/nut) to grade AAAAA (25.2 g/nut) (Hochmuth et al., 2013). In another scheme, grade A chestnuts, which were the smallest sized nuts, ranged from 8.3 to 10 g/nut (Chong, 2013).

If large-sized chestnuts are a production requirement, data from the present study indicate that crop load was excessive in the sixth year after grafting. Thus, thinning flowers or burs might be used at this time to enhance primary nut size at harvest. Several researchers thinned catkins in the spring with various growth regulators to adjust crop load and increase primary nut size at harvest in China (Su et al., 1998; Zhou et al., 2000; Zongyun and Kuiying, 2009). Warmund et al. (2005; 2010) also demonstrated that hand removal of secondary burs in late July or early August increased average primary nut weight at harvest. While several commercially-available thinning products labeled for fruit trees have been tested on chestnut in the U.S., none of these effectively removed secondary burs when applied in late summer (M.R. Warmund, unpublished data). Such thinning studies have been difficult to conduct due to natural abortion of secondary catkins when ambient temperatures exceed 37°C for five consecutive days in late July and early August.

In conclusion, ‘Little Giant’ slightly reduced tree size and did not affect cumulative yield when used as an interstem with an ‘AU-Super’ scion and AU-Cropper rootstock. However, ‘Little Giant’ interstem did not influence tree size or cumulative yield when grafted onto ‘Eaton’ and AU-Cropper rootstock. As annual yields generally increased throughout the study, average nut weights dropped in 2011 and remained low through 2013. Also, all trees exceeded their allotted space within the row in 2012 and trees had similar cumulative yield efficiency values in 2013. Thus, additional horticultural practices are needed to enhance cropping efficiency of chestnut trees and maintain nut size.

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