MORTALITY, EARLY GROWTH, AND BLIGHT OCCURRENCE IN HYBRID, CHINESE, AND AMERICAN CHESTNUT SEEDLINGS IN WEST VIRGINIA

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Abstract.—Two plantings of second (BC_3F_2) and third (BC_3F_3) backcross generations of hybrid American chestnuts established in east-central West Virginia were assessed after 4 years to determine family effects on growth and survival. Pure American and pure Chinese chestnut seedlings were also planted as controls for height growth, form, blight occurrence, and blight resistance. Overall mortality after four growing seasons totaled 12 percent and 41 percent on the two sites. In 2014, the mean height of live stems differed by site and by seedling type. Pure American and BC_3F_3 hybrids were the tallest on both sites. Changes in height from time of planting to 2014 were similar or the same for American seedlings and BC_3F_3 hybrids on both sites. A similar trend among seedling types occurred for total height; American seedlings and BC_3F_3 hybrids performed similarly and showed the greatest mean total height on each site compared to the other seedling types. Two BC_3F_3 families had greater than 25 percent of their stems considered poor form on the Cheat site; 45 percent of the stems in one BC_3F_3 family were considered poor form on the Gauley site in 2015. Considering seedling types only, the greatest percentage of stems with a canker rating of 4 (blight-killed stems) was found for the BC_3F_3 hybrids on the Gauley site. There is little incidence of stems with a rating of 3 (blight present, sunken surface of cankers, large area covered, fruiting bodies present) or 4 on the Cheat site compared to the Gauley site. Family differences in height, form, dieback/resprouting, and canker occurrence are becoming apparent and are expected to continue.

INTRODUCTION

The story of the American chestnut (*Castanea dentata*) with its past abundance and removal from the overstory because of chestnut blight (*Cryphonectria parasitica*) and ink disease (*Phytophthora cinnamomi*) is well documented (Crandall et al. 1945, Smith 2000, Youngs 2000). American chestnut now persists in the eastern forest as understory stump sprouts that sometimes reach reproductive age (Paillet 2002, Woods and Shanks 1959). Sites where American chestnut now grows may reflect a contraction of the species' niche having shifted to dry, southern- to western-facing slopes from more mesic sites as a result of the blight (Burke 2012). The loss of American chestnut from the overstory affected species composition. Northern red oak (*Quercus rubra*), chestnut oak (*Q. prinus*), and white oak (*Q. alba*) increased in overstory importance after the loss of American chestnut (Keever 1953), oaks and red maple (*Acer rubrum*) replaced American chestnut in gaps (Woods and Shanks 1959), and hemlock (*Tsuga canadensis*) and yellow-poplar (*Liriodendron tulipifera*) replaced American chestnut on more mesic sites (Elliott and Swank 2008). If diseased trees were not removed by logging, individual trees could take 2-10 years or longer to die from the blight, so in some areas, adjacent nonchestnut trees gradually filled in the resultant gaps (Woods and Shanks 1959).

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West Virginia is in the center of the species' former range (Little 1977), and American chestnut was noted to occur across the state, comprising 12 percent of the overstory by some estimates (Brooks 1910). Based on witness trees in land surveys, American chestnut was a relatively minor component of European-contact forests in north-central West Virginia; the importance values were 1.5, 1.7, and 2.4 in three counties (Rentch and Hicks 2005). Witness trees for the area that later became the Monongahela National Forest (MNF), also in West Virginia, show that about 6 percent of the trees listed in deeds were American chestnut; however, they were not evenly distributed, ranging from 4 percent to 18 percent of witness trees depending on ecological subsection (Thomas-Van Gundy and Strager 2012). American chestnuts were associated with moderate elevations, sites of low moisture, high topographic roughness, ridge landforms, and soils weathered from acid sandstone (Lily soil series); few American chestnuts grow in the highest elevations (Thomas-Van Gundy and Strager 2012).

Efforts to create blight-resistant or blight-tolerant American chestnut hybrids or clones started soon after the disease effects were felt (Anagnostakis 2012). The U.S. Department of Agriculture Bureau of Plant Industry began crossing American chestnut with Asian species to produce trees with American chestnut form and Asian resistance (Clapper 1954, Graves 1942). These efforts later became part of the Connecticut Agricultural Experiment Station's work on restoring American chestnut (Anagnostakis 2012). The backcross method of breeding was introduced into these efforts in 1986 (Burnham et al. 1986). The American Chestnut Foundation (TACF), founded in 1983, began with the mission to backcross blight resistance from the Chinese chestnut (*C. mollissima*) into the American chestnut (Ellingboe 1994, Hebard 2001). The latest hybrid available from TACF (BC_3F_3) is predicted to be approximately 94 percent American chestnut (Ellingboe 1994, Hebard 2001). The BC_3F_3 hybrids are the third generation of the third backcross to produce trees that are predicted to have blight resistance similar to the Asian chestnut and the "timber" form of the American chestnut (Hebard 2001). Resistance testing of the parents of these hybrids suggests that blight resistance is likely to be intermediate to high (Hebard et al. 2014). Seedlings of the recent cross, BC_3F_3 "restoration" chestnut, are showing levels of blight resistance that are intermediate between the resistance of pure American chestnut and the Chinese breeding stock (Hebard 2012).

Before the blight, American chestnut in the southern Appalachian Mountains grew faster than most other associated species, reaching half their final height by age 20. Only white pine (*Pinus strobus*) and yellow-poplar grew faster (Ashe 1912). Stump sprouts in Connecticut reached 10 feet by age 4 and were the tallest sprouts in the hardwood coppice forest (Schwartz 1907). Reproduction through the formation of seedling-sprouts was described for one area as sprouts occurring at the base of the stem or just below the soil surface on 12- to 18-year-old seedlings; these could persist for 30–60 years with intervening cycles of dieback from shading or browse (Schwartz 1907). Based on stump sprouting ability (American chestnut is not known to root sprout [Schwartz 1907]) the creation of even-aged stands through coppice cutting was recommended for managing American chestnut (Zon 1904).

In 2011, the Northern Research Station of the U.S. Forest Service, the MNF, and TACF began cooperating on a long-term test planting of BC_3F_3 hybrids on national forest lands as part of a broader effort (Clark et al. 2014a). Along with outplantings, the hybrids are being screened for blight resistance by TACF at their seed orchards, and families that do not show resistance are removed from further breeding schemes (Hebard 2012). Hybrid seedlings on the MNF were planted to determine: (1) the degree to which the BC_3F_3 seedlings resemble American chestnuts in a natural forest setting and the degree to which Chinese characteristics (other than blight resistance) remain (Hebard et al. 2014), (2) the degree to which the BC_3F_3 seedlings are resistant to blight, and (3) how long resistance persists in these hybrids. In this analysis, we report the 4-year results on mortality, height growth, occurrence of resprouting, and occurrence of blight.

METHODS

Study Area

Two sites on the MNF were chosen for planting: one on the Cheat Ranger District and one on the Gauley Ranger District. The Cheat site is 7 acres that were part of a commercial timber sale, fenced to prevent deer browsing after clearcut harvest in 2008, and located in Tucker County near the town of Saint George, WV (39°08'56.7" N, 79°41' 50.1" W). Site preparation after harvest included severing all stems shorter than 2 inches diameter at breast height except desirable tree regeneration. Some red maple stump sprouts were treated with 2 percent RazorPro® herbicide in fall 2010. Herbicide was released on the planted seedlings in fall 2011. Individual planted seedlings were released from woody stem competition within a 2- to 3-foot radius by application of RazorPro. Blackberry (*Rubus* spp.) stems were treated only if they completely overtopped the seedling.

The Gauley site is a 2-acre portion of a larger clearcut located in Webster County above Sawyer Run, a tributary to the Williams River near the town of Cowen, WV (38°23'57.8" N, 80°26' 28.4" W). The planted area was harvested and fenced in 2010. Treetops and logging slash resulting from timber harvest were removed from the site before planting. The remainder of the 37-acre clearcut was harvested in 2011. Vis-pore® mats were used to control competing vegetation as trees were planted. Planted seedlings were released from overtopping competition by hand cutting selected stems in spring 2013. American chestnut saplings were noted on the site before harvest and planting.

The Gauley plantation site is higher in elevation (3,280-3,300 feet) than the Cheat site (2,460-2,500 feet). Both sites have moderate site indices (index species of northern red oak) at 72 for the Gauley site and 75 for the Cheat. The Gauley site faces mainly southwest and the Cheat site faces northwest. Soils on the Gauley site are mapped as the Clifftop-Laidig association, and the Cheat site is classified as Gilpin channery silt loam in the respective county soil surveys. Surface soil on the Gauley planting site appears higher in sand content than the Cheat site, and the site includes sandstone surface boulders.

Both sites were tested for infection by *Phytophthora* root rot (*P. cinnamomi*), and samples showed no infection at the time of planting. *P. cinnamomi* has caused American chestnut mortality, particularly at low elevations, starting in the 1820s when it was accidently imported from Asia (Anagnostakis 2012). American chestnut is highly susceptible to *Phytophthora* root rot even on moderately wet soils with little compaction (Clark et al. 2014b, Rhodes et al. 2003); therefore, this pathogen must not be present at the start of the study.

Study Design and Data Collection

Nursery stock was planted by contractors (Gauley) or MNF staff (Cheat) using standard hand planting techniques (planting bars) and power augers. Seedlings were planted in March and April 2011 in rows about 8 feet apart with about 8 feet between seedlings as site conditions allowed. The seedlings were 1-year-old bare-root stock that had been grown at the Virginia State nursery near Waynesboro, VA. The BC_3F_3 seedlings were grown from nuts produced in seed orchards at TACF's farms near Meadowview, VA. The hybrid seedlings were open pollinated; family designations are from the TACF breeding scheme where families share a single mother tree of known genetics. Most of the American chestnut ancestors of the trees in the seed orchard were in the vicinity of Mount Rogers National Recreation Area, VA, but some originated further south in the Appalachian Mountains. Mount Rogers National Recreation Area is approximately

140 miles from the Gauley planting site, about 200 miles from the Cheat planting site, and 2°-3° of latitude south of the two planting sites; therefore, we assumed the experimental material from the BC_3F_3 and American chestnut were locally adapted to the MNF study areas.

Plantation studies require that mortality of individuals be considered when setting up the experimental design. The planting design needs to include enough experimental units such that data collected can be assessed with statistical power. Resolvable incomplete block design (Patterson and Williams 1976) was chosen for this study to address the concerns of expected mortality, the number of families to test, and differences in planting site conditions. Each incomplete block contained 35 trees.

Seedlings from 23 families of BC_3F_3 seedlings were planted at each site (Table 1), but not all families were planted at both sites. Thirty-one families of BC_3F_3 seedlings were planted across

Table 1.—Family names, numbers of trees, and the type of seedlings planted on each site. Gray highlights show families planted at both sites.

Cheat		Gauley		
Family	Count	Family	Count	Type
BLWPureAmerican	25	BLWPureAmerican	25	Pure American
CliffAcc	25	CliffAcc	25	Pure American
HaunAmerican	25	HaunAmerican	25	Pure American
CH297	25	CH297	25	BC_3F_2
SC80	25	SC80	25	BC_3F_2
D1-28-138	50	D1-28-138	50	BC_3F_3
D2-26-72	25	D2-26-72	25	BC_3F_3
D2-28-52	25	D2-28-125	25	BC_3F_3
D2-29-27	25	D2-28-42	25	BC_3F_3
D2-50-15	21	D2-28-52	26	BC_3F_3
D3-27-105	24	D2-50-12	25	BC_3F_3
D3-27-46	24	D2-50-15	24	BC_3F_3
D4-18-30	25	D3-27-105	25	BC_3F_3
D4-20-65	25	D3-27-46	25	BC_3F_3
D4-26-63	25	D4-18-30	25	BC_3F_3
D4-27-103	25	D4-20-65	25	BC_3F_3
D5-17-130	50	D4-26-63	25	BC_3F_3
D5-17-61	50	D5-17-130	50	BC_3F_3
D5-17-89	25	D5-17-61	50	BC_3F_3
D5-25-49	26	D5-17-89	25	BC_3F_3
D5-26-54	49	D5-25-147	25	BC_3F_3
D5-27-101	24	D5-26-54	50	BC_3F_3
D7-26-86	50	D6-26-29	25	BC_3F_3
D8-26-104	25	D7-26-20	25	BC_3F_3
D9-26-36	25	D7-26-86	50	BC_3F_3
W1-29-8	24	D7-28-83	25	BC_3F_3
W1-31-63	25	D8-26-15	25	BC_3F_3
W3-32-49	25	W1-31-63	25	BC_3F_3
opCD	49	opCD	50	Pure Chinese
Grand total	866		875	

both sites. Seedlings of pure American chestnut (three families at both sites), Chinese chestnut (one family at both sites), and intermediate hybrids (BC_3F_2; two families on both sites) were planted as control trees. Approximately 875 trees were planted at each site (Table 1). Each seedling was assigned an individual numbered tag to ensure that family identification and initial seedling characteristics could be followed throughout the study.

Initial heights were recorded within 2 weeks of planting in 2011. Total height (base of the tree to the top of the tallest live bud) was measured in early spring 2012, 2013, 2014, and 2015 on the Cheat site and early spring 2012, 2013, and 2015 on the Gauley site. Survival, damage, and form were noted for each seedling using qualitative descriptions when heights were taken. Damage remarks included dead top resprouted, dead top, resprouted, insect damage, bear damage, broken top, herbicide damage, top cut at nursery, overtopped, live/dead, yellow leaves (not related to fall color change), bent stem, grapevine on stem, overtopped by slash, and greenbrier on stem. Stems were considered to have poor form if any of the following were noted in 2015: epicormic branching, forking, low forking, lean (not related to storm damage), multistem, flat top, or crook. By 2015, blight cankers were evident on some stems and severity class was noted. Classes used were 1 (no blight); 2 (blight present but superficial or swollen, no fruiting bodies); 3 (blight present, sunken surface of cankers, large area covered, fruiting bodies present; and 4 (blight-killed stem). These ratings were adopted from a similar study conducted by the Southern Research Station (S. Clark, personal communication).

Data Analysis

Differences in performance among families were analyzed as generalized linear mixed models with live seedling height assessed three ways: (1) mean height in 2014 (no resprouting individuals included), (2) mean change in height from the time of planting to 2014 (no resprouting individuals included), and (3) mean total height from 2011 through 2014 (resprouts included and time as repeated measure). Sites were assessed separately. For the first two measures, differences in performance among families were analyzed as generalized linear mixed models (PROC GLIMMIX, SAS 2012) with the intercept as a random variable and the family as the fixed variable. These models were run as incomplete block design using a gamma distribution with log link function on the Cheat site and Gaussian response distribution with the identity link on the Gauley site. For both sites, denominator degrees of freedom were calculated with the Kenward-Rodger method. For these analyses, significance was tested at $\alpha = 0.05$, pairwise comparisons were adjusted using the Tukey Kramer method, and back transformed least square means for family are reported.

Calculation of mean total height (the third height measure) did include stems that had resprouted during the 4 years. The differences among families were analyzed as generalized linear mixed models with repeated measures (PROC GLIMMIX, SAS 2012) with initial height (2011) as covariate following methods in Littell et al. (1996). Models were run as incomplete block design using a gamma distribution with log link function with family and year and family*year interaction tested; year and intercept were random variables. For both sites, denominator degrees of freedom were calculated with the Kenward-Rodger method. For these analyses, significance was tested at $\alpha = 0.05$, pairwise comparisons were adjusted using the Tukey Kramer method, and back transformed least square means are reported.

Descriptive statistics were used for percentage of mortality at 2015, percentage of resprouting in any year, and percentage of canker rating class at 2015, all by family and site.

RESULTS

Mortality

Mortality after the first growing season (seedlings surveyed in October 2011) differed greatly by site at about 1.6 percent for the Cheat sites and about 12.7 percent for the Gauley site (data not shown) for all seedling types. By the end of the 2013 growing season, mortality on the Gauley site had increased to 23.9 percent and by only 7.3 percent on the Cheat site. Mortality after 4 seasons and across all families totaled 12 percent on the Cheat site and 41 percent on the Gauley site (Fig. 1). About 13 percent of the BC_3F_3 hybrids planted on the Cheat site had

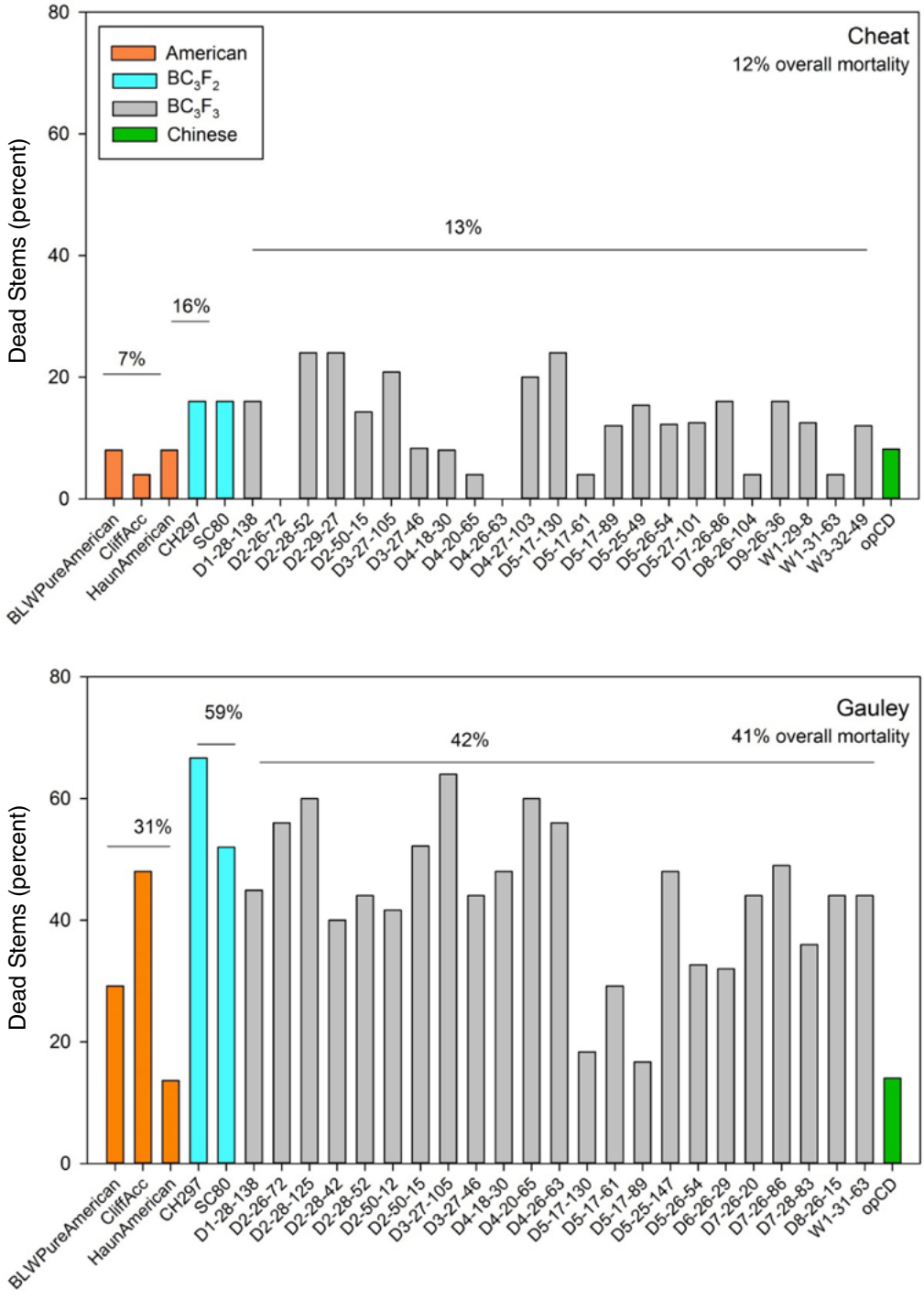


Figure 1.—Mortality by family as of spring 2015. Percentages given above vertical lines are the mean mortality by seedling type.

died compared to 42 percent on the Gauley site. No families experienced greater than 50 percent mortality on the Cheat site, and 10 families experienced that level or higher on the Gauley site. On the Cheat site, the highest mortality occurred in the BC_3F_3 families (D2-28-52, D2-29-27, and D5-17-130) at 24 percent of seedlings for each family. As a group, pure American chestnut families had the lowest mortality at 7 percent compared to 16 percent for the BC_3F_2 hybrids and 8 percent for pure Chinese on the Cheat site. On the Cheat site, 2 families, D2-26-72 and D4-26-63, showed no mortality. Mortality was higher in all families and groups on the Gauley site; 31 percent of the pure American chestnut were dead by 2015, as were 59 percent of the BC_3F_2, 42 percent of the BC_3F_3, and 14 percent of the pure Chinese seedlings.

Height

In 2014, the mean height of live stems (no resprouting stems included) differed by site and by seedling type. Pure American and BC_3F_3 hybrids were tallest on both sites (Table 2). BC_3F_3 hybrids averaged 9.3 ± 0.4 feet (mean ± standard error) on the Cheat site and 7.6 feet (± 0.2 feet) on the Gauley site. Seedlings of pure Chinese origin were shortest on both sites. The greatest mean heights in 2014 were found in three BC_3F_3 families on the Cheat site: D2-26-72 (10.9 feet ± 0.8 feet), D4-18-30 (10.1 feet ± 0.8 feet), and D5-26-54 (10 feet ± 0.6 feet), although their mean heights differ significantly from only one BC_3F_3 family (D5-17-130, 6.7 feet ± 0.4 feet) and the Chinese chestnut family (Fig. 2). On the Gauley site no significant differences were found among families; however, the greatest mean height was 8.7 ± 0.8 feet for seedlings of the D2-28-125 family (Fig. 2).

Table 2.—Arithmetic means and standard errors (SE) for the three measures of height by site and seedling type

	Cheat		Gauley	
Type	Mean	SE	Mean	SE
	Height in 2014 (feet)			
Pure American	9.3	0.2	7.5	0.2
BC_3F_2	8.0	0.4	6.1	0.3
BC_3F_3	9.3	0.2	7.6	0.2
Pure Chinese	7.1		5.9	
	Change in height from 2011 to 2014 (feet)			
Pure American	6.0	0.0	4.8	0.3
BC_3F_2	5.0	0.0	5.1	0.6
BC_3F_3	6.0	0.1	4.6	0.2
Pure Chinese	4.6		3.7	
	Total height growth (feet)			
Pure American	5.8	0.0	5.2	0.2
BC_3F_2	4.9	0.1	4.3	0.3
BC_3F_3	5.8	0.1	5.5	0.1
Pure Chinese	4.3		3.8	

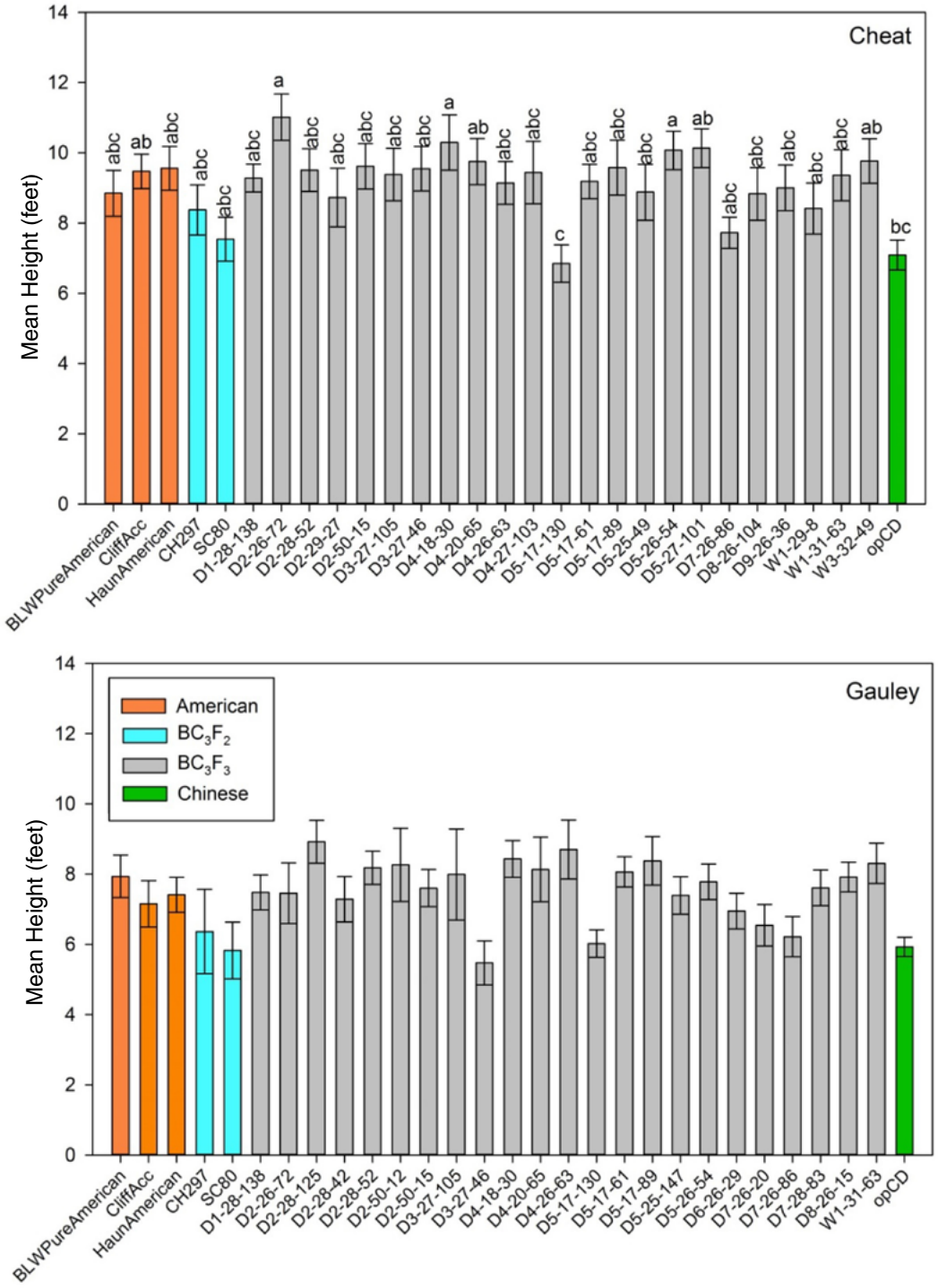


Figure 2.—Least square mean heights (± SE) by family in 2014. Means with different letters are significantly different (α = 0.5); no significant differences were found for the seedlings on the Gauley site.

As found for mean height in 2014, the change in height from time of planting to 2014 (no resprouting stems included) was similar or the same for pure American seedlings and BC_3F_3 hybrids on both sites (Table 2). Greater variability in this measurement on the Gauley site resulted in no statistically significant differences among families (Fig. 3). On the Gauley site, seedlings in the D2-28-125 family (6.3 ± 0.6 feet) were significantly taller than those in the D3-27-46 (3 ± 0.6 feet) and D5-17-130 (3.1 ± 0.3 feet) families (all BC_3F_3). The greatest mean change in height for the Cheat site was found in the BC_3F_3 family D2-26-72 (7.3 ± 1.5 feet), which also showed no mortality. On the Gauley site, the greatest mean change in height also occurred in a BC_3F_3 family, D2-28-125, which also had the greatest mean height in 2014. On both sites, one BC_3F_3 family had mean height growth at or lower than the pure Chinese chestnut trees, D5-17-130.

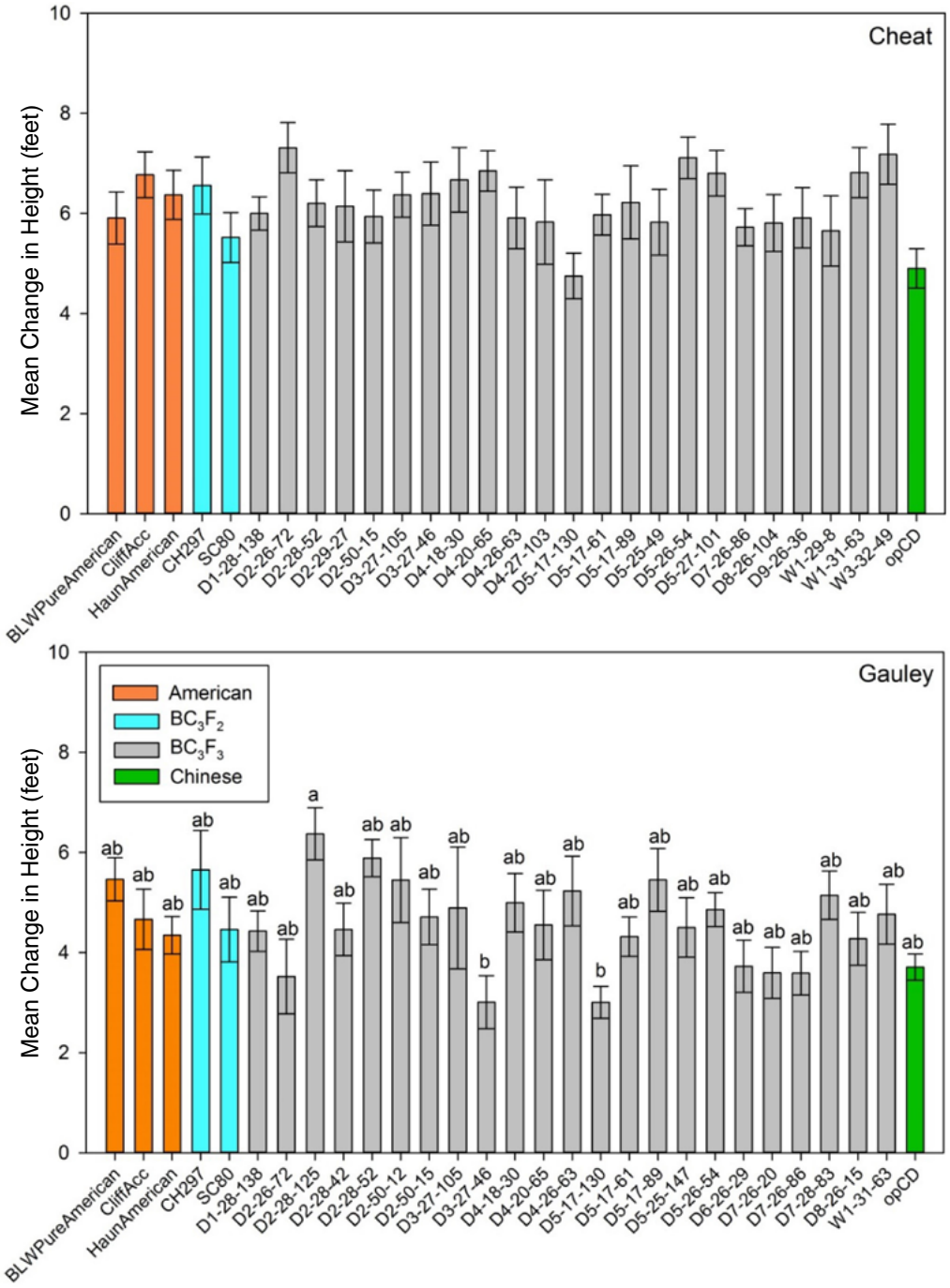


Figure 3.—Least square mean change in height from 2011 to 2014 (± SE) by family. Means with different letters are significantly different (α = 0.5); no significant differences were found for seedlings on the Cheat site.

A similar trend among seedling types is found for total height growth. Pure American seedlings and BC_3F_3 hybrids performed similarly and showed greatest mean total height compared to the other seedling types (Table 2). When total height growth over time is tested, including resprouting individuals, family differences are statistically significant (Fig. 4). On the Cheat site, six families (one pure American and five BC_3F_3) had greater growth than one family (BC_3F_3), and on the Gauley site, one family (BC_3F_3) showed significantly greater growth over four families (two BC_3F_3 families, one BC_3F_2 family, and one pure Chinese family). Seedlings of the D5-17-130 hybrid family showed generally lower total growth at both sites (lowest on the Cheat site), although this was not statistically different than many other families. Similarly, pure Chinese chestnut seedlings on the Gauley site showed generally less total height growth, significantly less than 14 out of 29 families.

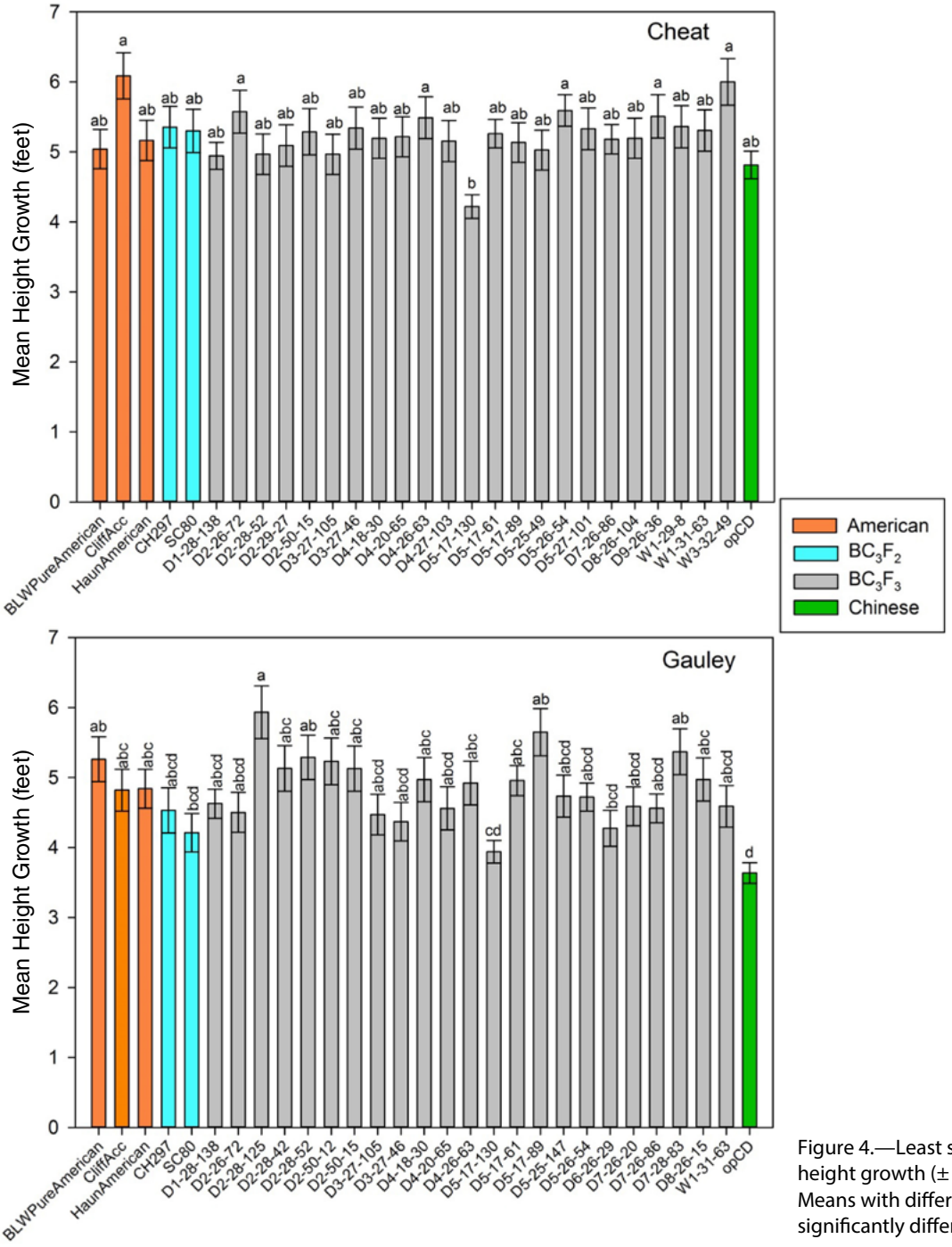


Figure 4.—Least square mean total height growth (± SE) by family. Means with different letters are significantly different (α = 0.5).

Form and Canker Incidence

The number of stems considered to have poor form in 2015 was generally higher for all seedling types on the Gauley site compared to the Cheat site (Table 3). Two BC_3F_3 families had greater than 25 percent of their stems considered poor form on the Cheat site (D4-26-63 and D5-17-89); 45 percent of the stems in one BC_3F_3 family (D5-17-61) were considered poor form on the Gauley site in 2015 (Fig. 5). One family on the Cheat site (D2-28-52) and two on the Gauley site (D2-26-72 and D3-27-105) showed no stems considered poor form in 2015. The lowest incidence of poor form was found in the pure Chinese seedlings on the Cheat site.

Table 3.—Mean percentage of stems with poor form (in 2015) by site and seedling type

	Cheat	Gauley
Type	% of stems	% of stems
Pure American	9.3	16.7
BC_3F_2	6.0	22.9
BC_3F_3	9.2	18.2
Pure Chinese	2.0	23.3

Pure Chinese seedlings have the greatest incidence of dieback and resprout at both sites (Table 4; about 25 percent of stems on the Cheat site and 54 percent on the Gauley site). The incidence of main stem dieback with resprouting, noted in the remarks for each seedling, was also assessed by family (Fig. 6). The BC_3F_3 hybrid with low height growth (D5-17-130) showed resprouting on about 20 percent (Cheat site) and 28 percent (Gauley site) of stems.

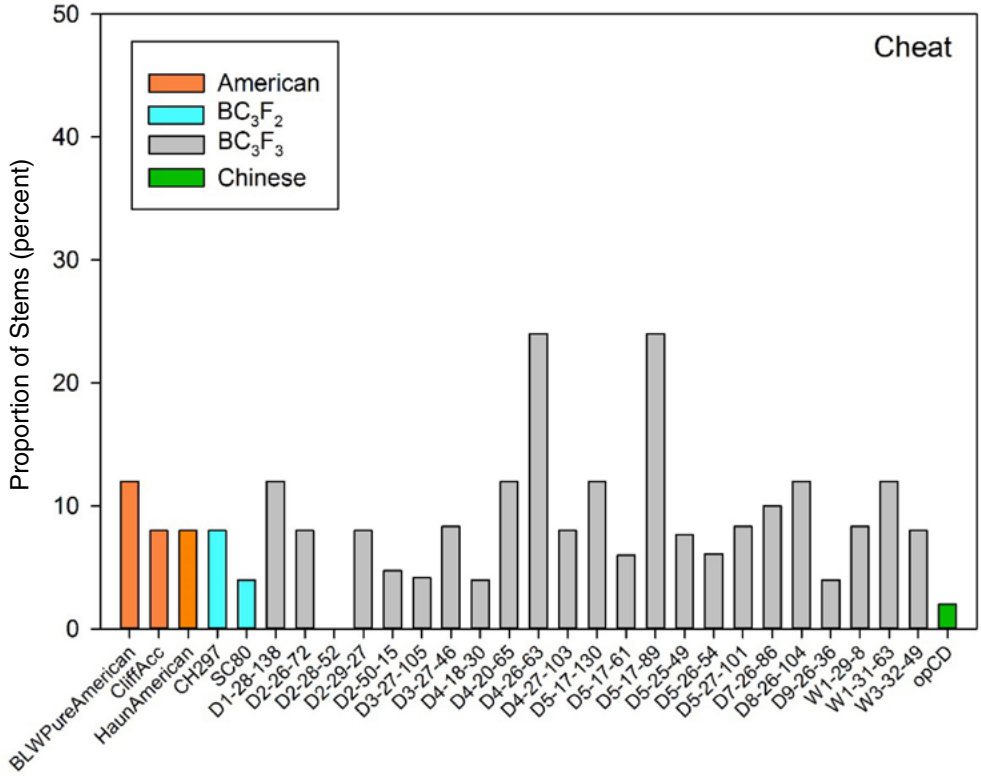
Table 4.—Mean percentage of stems that resprouted from dieback any year by site and seedling type

	Cheat	Gauley
Type	% of stems	% of stems
Pure American	6.7	12.2
BC_3F_2	6.0	8.0
BC_3F_3	8.9	11.4
Pure Chinese	24.5	54.0

Only the percentages of stems with ratings of 2, 3, or 4 are reported here because a 1 rating meant no cankers noted. Considering seedling types only, the highest percentage of stems with canker rating of 4 (blight-killed stems) was found for the BC_3F_3 hybrids on the Gauley site (Table 5). Few stems had a rating of 3 (blight present, sunken surface of cankers, large area covered, fruiting bodies present) or 4 on the Cheat site compared to the Gauley site (Fig. 7). Two BC_3F_3 families on the Cheat site show 17 percent and 19 percent of stems with a canker rating of 2 (D4-18-30 and W1-29-8, respectively), and these are the highest percentages of 2 ratings on this site. Three BC_3F_3 families on the Gauley site show 20 percent, 20 percent, and 17 percent of stems with canker rating of 2 (D3-27-105, D5-17-89, and D5-26-54, respectively). Only 5 BC_3F_3 families on the Cheat show stems that were considered killed by blight (4 rating) ranging from about 3 percent to 9 percent of stems, and no American chestnuts had rankings higher than 3. In comparison, on the Gauley site 24 families had stems killed by blight, ranging from about 2 percent to 29 percent of stems.

Table 5.—Mean percentage of stems with canker rankings of 2, 3, or 4 by site and seedling type

	Cheat			Gauley		
	% of stems			% of stems		
	Canker rating			Canker rating		
Type	2	3	4	2	3	4
Pure American	4.3	1.4	0.0	5.4	5.2	7.9
BC_3F_2	4.8	0.0	0.0	9.4	3.8	9.4
BC_3F_3	4.9	0.8	1.1	7.9	2.9	10.7
Pure Chinese	11.1	0.0	0.0	11.6	7.0	0.0



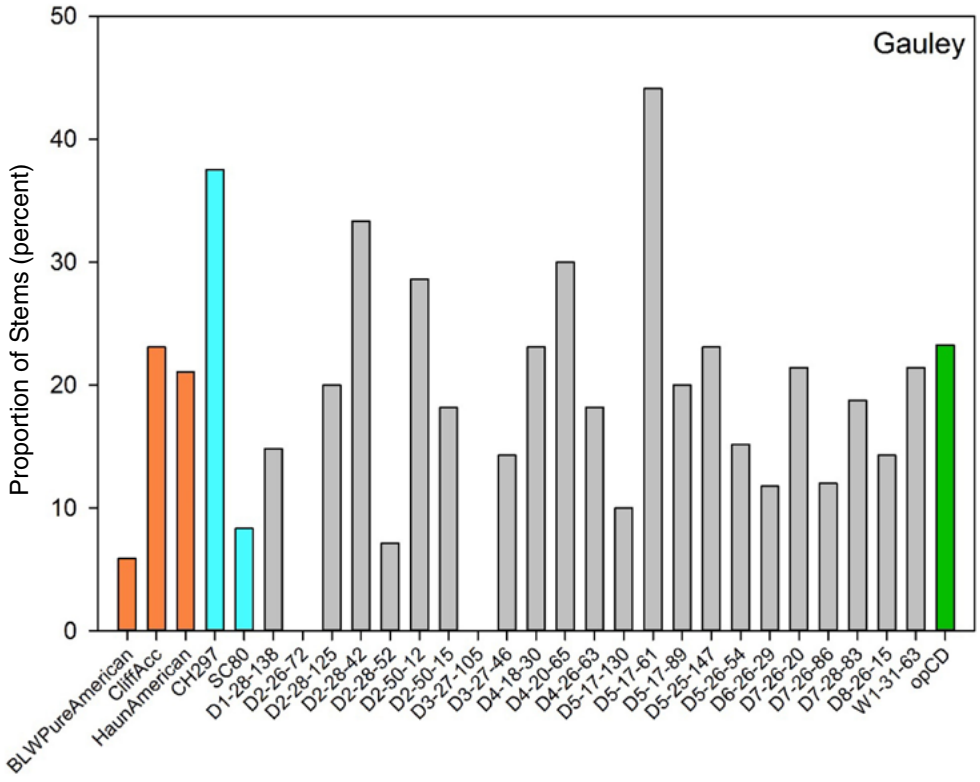


Figure 5.—Percentage of stems with poor form (in 2015) by family.

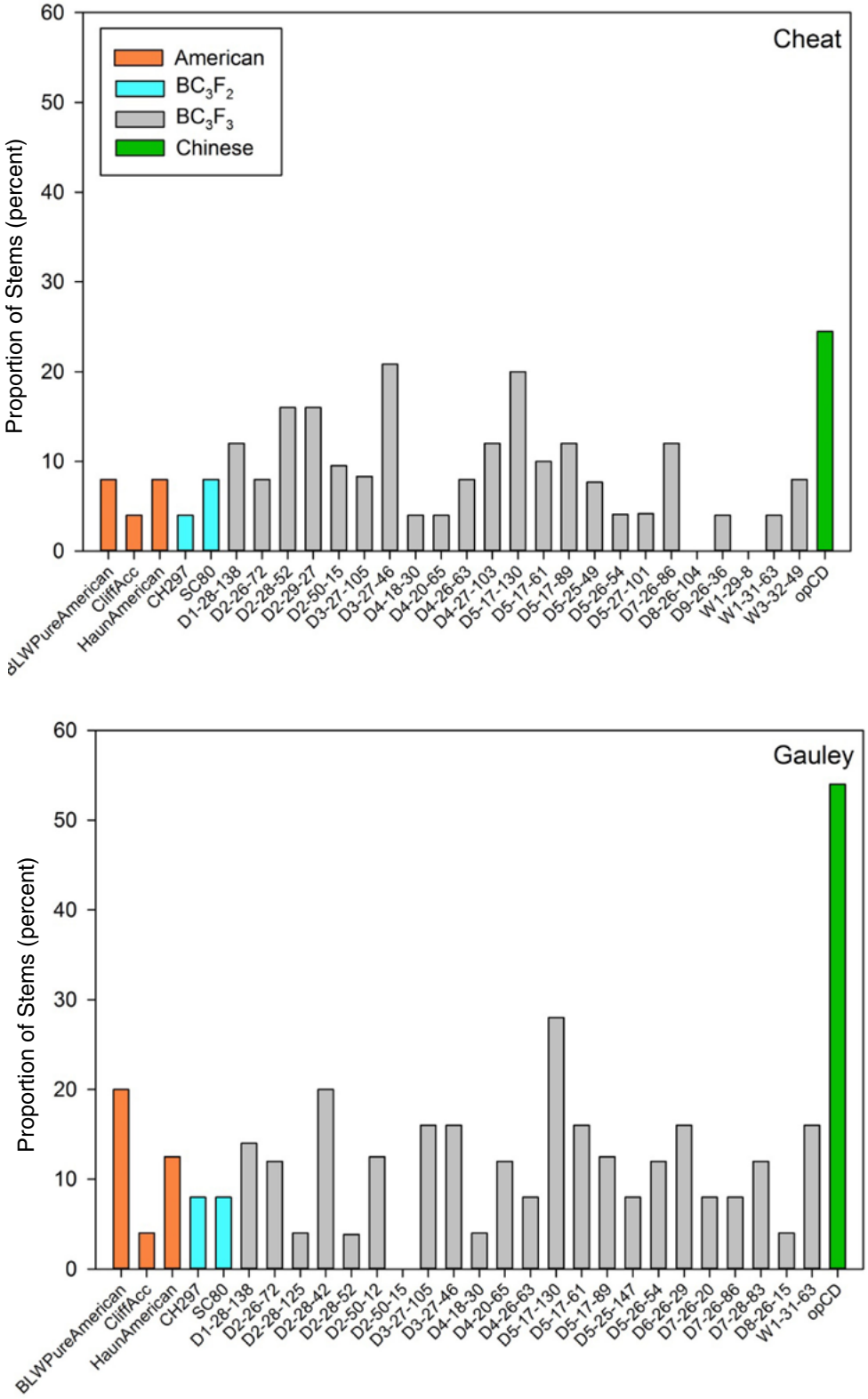


Figure 6.—Incidence of resprouting in any year by family.

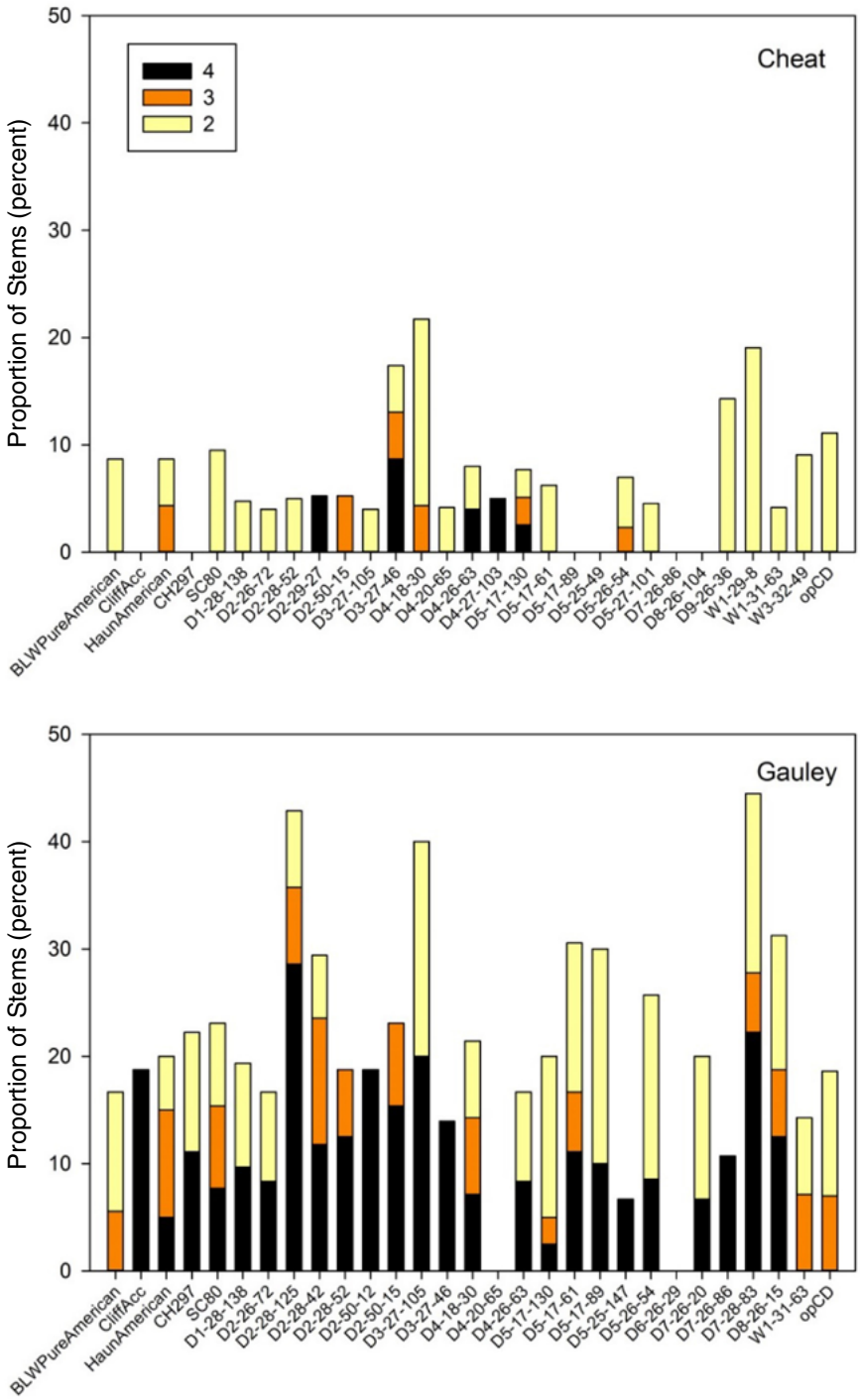


Figure 7.—Canker ratings (for ratings 2-4 only) in spring 2015 by family. Ratings are 2: blight present, superficial, swollen, no fruiting bodies; 3: blight present, sunken surface of cankers, large area covered, fruiting bodies present; 4: blight-killed stem.

DISCUSSION

As Clark et al. (2015) found in plantings in North Carolina, Tennessee, and Virginia, the BC_3F_3 seedlings planted in West Virginia responded more like American chestnut than Chinese chestnut. Progeny and provenance studies on other Fagaceae species show that growth rates by family change as the individuals move from juvenile to adult stages (Kriebel et al. 1988, Míguez-Soto and Fernández-López 2015). We anticipate changes in the rankings of families over time at our sites for many measures, especially as the stands reach the stem exclusion stage of development.

The spatial patterns of mortality on the Gauley site, especially within 1 year of planting, suggest that some combination of compaction, soil moisture, and perhaps *P. cinnamomi* compromised seedling growth. In 2012, an unknown species of *Phytophthora* was found on the Gauley site; further testing of soils and dead seedlings is needed to confirm the presence of *P. cinnamomi*. We do know, however, that ink disease is present at the nursery that produced the seedlings we used.

Treetops were skidded off the Gauley site immediately before planting. Skidder paths were visible as lanes through the site. Most seedlings planted in these lanes did not survive to age 4. In a greenhouse study, American chestnut seedling mortality was highest on wet and compacted soils regardless of fungicide treatment (Rhoades et al. 2003). In a study similar to ours with hybrid seedlings planted in forested conditions, higher mortality occurred on sites with poor drainage (Clark et al. 2014b). Although the numbers of seedlings in the greenhouse study were low, the results emphasize the findings of others that American chestnut seedlings are not well suited to wet and compacted soils. Based on our field observations, this also holds true for the advanced hybrid seedlings.

Another possible contributing factor to mortality on the Gauley site is soil texture. Mortality in a planted stand of American chestnuts in eastern Kentucky was negatively related to sand and coarse fragment content in the soil (Rhoades et al. 2009), where the percentage of sand and coarse fragments (measured and assessed separately) was negatively related to seedling survival. The Clifftop-Laidig soil association underlying the Gauley site is described as extremely stony, which applies to the surface, and a channery silt loam in the A horizon (1-3 inches). By definition, in a channery silt loam, 15–35 percent of soil volume is in channers, flat rock fragments up to 6 inches long. Using that composition range and the relationship between coarse fragments and seedling survival described by Rhoades et al. (2009), survival on the Gauley site is predicted as approximately 50-74 percent; year 4 survival on the Gauley site was 59 percent.

The poor form category included low forks, epicormic branching, and lean (not related to storm damage). Given storm damage on the Cheat site (Hurricane Sandy, snow-on-leaves event in October 2012), it was surprising that the percentage of stems with poor form was not higher. In this storm, blackberry thickets that still had leaves trapped snow and bent or broke many seedlings that were growing underneath the blackberry. Some stems did break off completely; however, those would not have been in the poor form category. About 450 stems were staked after the storm; many stakes and twine had rotted off by 2015. Temporary staking may have confounded the use of this qualitative measure for determining desirable families to continue in the breeding program. The staking of at least some of the worst-affected stems was considered necessary to salvage the study.

Sprouting is part of the reproduction strategy of American chestnut (Paillet 2002, Russell 1987, Schwartz 1907, Wang et al. 2013) but can confound height growth measurements. Resprouting could be in response to planting shock, dieback from blight or another disease, or damage from small mammals. For these reasons, height growth was expressed in three ways. Only the total

height growth over the entire 4 years (Fig. 4) included individuals that resprouted; for the other two measurements, resprouted individuals were removed from calculations. Although the incidence of resprouting after dieback (Fig. 6) may not be directly used to determine successful families for restoration planting, it will be interesting to see if any families are consistent resprouters. Others have found dieback to be greater in seedlings of larger ground-line diameter and for Chinese chestnuts, although no differences among hybrid families were found (Clark et al. 2015).

These results represent only 1 year of rating stems for the occurrence of chestnut blight cankers. It is likely too early to start removing families based on this criterion alone, although these families are also being screened at TACF orchards. Preliminary results of TACF screening efforts show that the D5-17-130 family shows resistance to the blight (J. Westbrook, personal communication); however, it is one of the shortest families in the West Virginia plantings at 6.7 feet on the Cheat site and 6.1 feet on the Gauley site in 2014. In terms of canker ratings in 2015 on these sites, family D4-18-30 shows a high percentage of stems with a canker rating of 2 on the Cheat site (Fig. 7), and TACF trials have also found high resistance to the blight fungus in this family (J. Westbrook, personal communication). TACF trials have also found seedlings of the D2-28-125 and D3-27-46 families to be low in resistance (J. Westbrook, personal communication). Our results show that seedlings of D2-28-125 have high mortality from blight on the Gauley site (Fig. 7) and the D3-27-46 family has higher mortality on the Cheat site.

This study is part of the larger goal of restoring American chestnut, as a self-regenerating hybrid, to forested settings where the species can continue to evolve (Hebard 2012). The number of backcross families under evaluation at our study sites is unique. These plantings will continue to be monitored for health and growth with the goal of informing the breeding program.

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