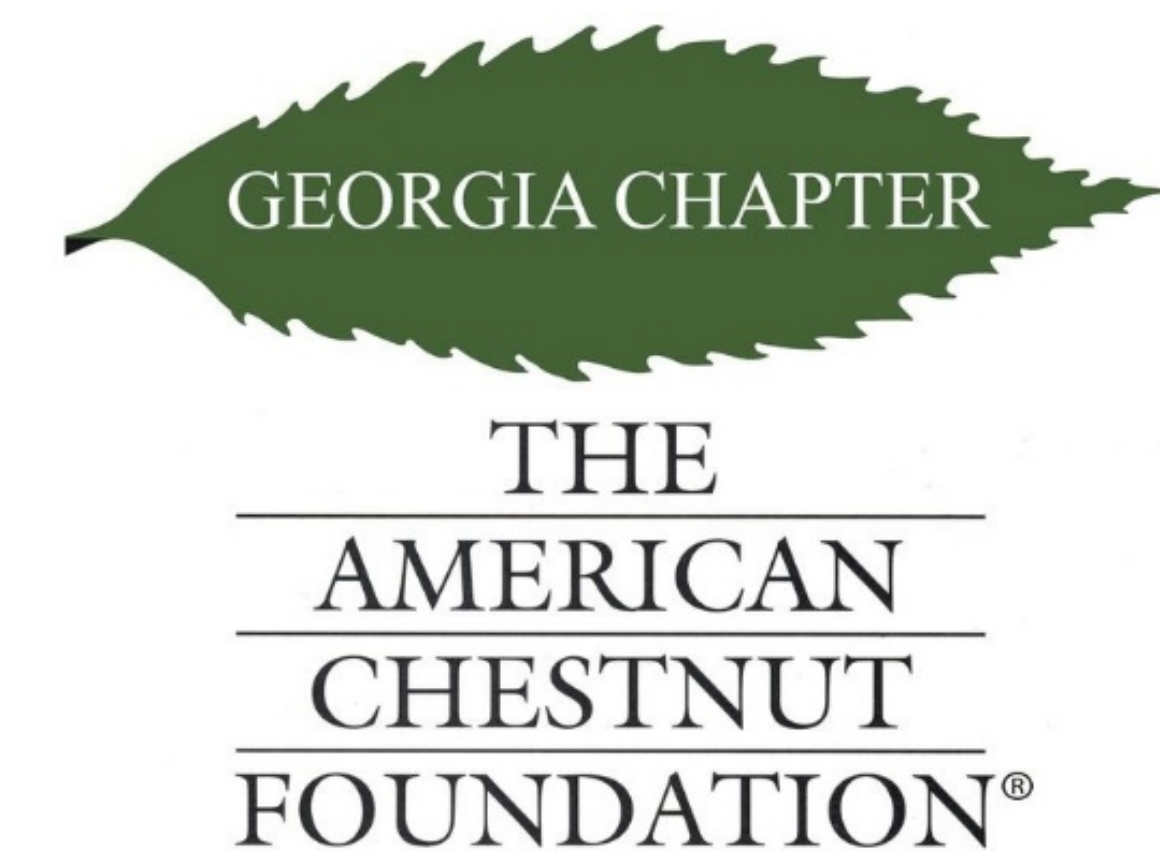




Evaluation of an alternative small stem assay in backcross chestnut seedlings in Georgia

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Introduction: We evaluated an alternative “small stem assay” (AltSSA) for blight resistance in backcross hybrid chestnut (*Castanea dentata/mollissima*) seedlings. Intended primarily for forward selection, this AltSSA was designed to be easy to implement and reproduce, to consistently induce cankering, and to better enable seedling recovery via the development of lateral shoots from the lower stem. In this analysis, we evaluated the relationship between AltSSA performance in offspring of backcross families, and predictions derived from blight resistance phenotype (two methods) and percent American (AM) chestnut ancestry of the parents.

Methods : Whereas standard SSAs are done by inoculating vertical incisions in the stem, our assays were done by cutting off ~4-5 mm diameter stems, inoculating the cut ends with disks of *Cryphonectria parasitica* inoculum, and covering them for one week with plastic sleeves. At 90+ days after inoculation, full canker length (FCL) and orange zone canker length (OZCL) were measured (Fig.1), and several other potential indicators of blight resistance were also scored, after which stem tips were cut ~2 cm below the cankers and disinfected to prepare seedlings for out-planting (Fig. 2).

Data Analysis

To evaluate the relative importance of variables used to assess blight resistance, we used principal component analysis (PCA) using correlation matrices with seedling and parental data separately. Additionally, a genetic-based blight resistance index was developed for parent trees and proportion of AM chestnut ancestry was also estimated for the parents of each line.

Figure. 1
Comparison of orange zone (O) and full (F) canker length between seedlings of susceptible-type (left) and resistant-type (right) after inoculation via the AltSSA method.



Figure. 2
Seedling after removal of canker and disinfection with a misting of 70% ethanol.



Results: In both years, canker production was near 100%, and average canker length for backcross trees did not differ appreciably between the two years (0.52 mm/day for EP in 2020 and 0.57 mm/day for Weekly in 2021).

Seedling data

When measurements of resistance-associated phenotypes were entered into a PCA, the first axis (PC1) explained 56.5% (2020) and 37.5% (2021) of the overall variation. Family mean OZCL was strongly related ($R^2 = 0.87$ (2020), $R^2 = 0.94$ (2021)) with the mean PCA-based blight resistance index for both years, and importantly the family means for both these variables indicate the range of blight resistance values expected of backcross lines, i.e. between F_1 and AM-level resistance.

Relationships with parental data

Analysis of parental blight resistance data via multi-factor PCA, showed that the first axis (PC1) accounted for 38.5% of overall variation, thus PC1 was used as one blight resistance index for parents. Significant relationships were apparent between the mean OZCL for the AltSSA seedlings and the PC1-derived resistance index of parents (Fig.3), the AM chestnut ancestry of parents (Fig. 5), and the genetic-based blight resistance index of parents (Fig. 4). Together, these relationships indicate agreement between the AltSSA and predictions based on parental phenotypes and genotypes.

Out-planting: In both years ~50% of seedlings were selected for out-planting based primarily on OZCL. In February of the years following inoculation, seedlings were planted in an orchard site near Ellijay, Georgia. Survivorship and evidence of blight were accessed during follow up visits to the orchard (Fig. 6). In May 2022, it was found that survivorship was high with 77% (2020) and 70% (2021) survivorship.

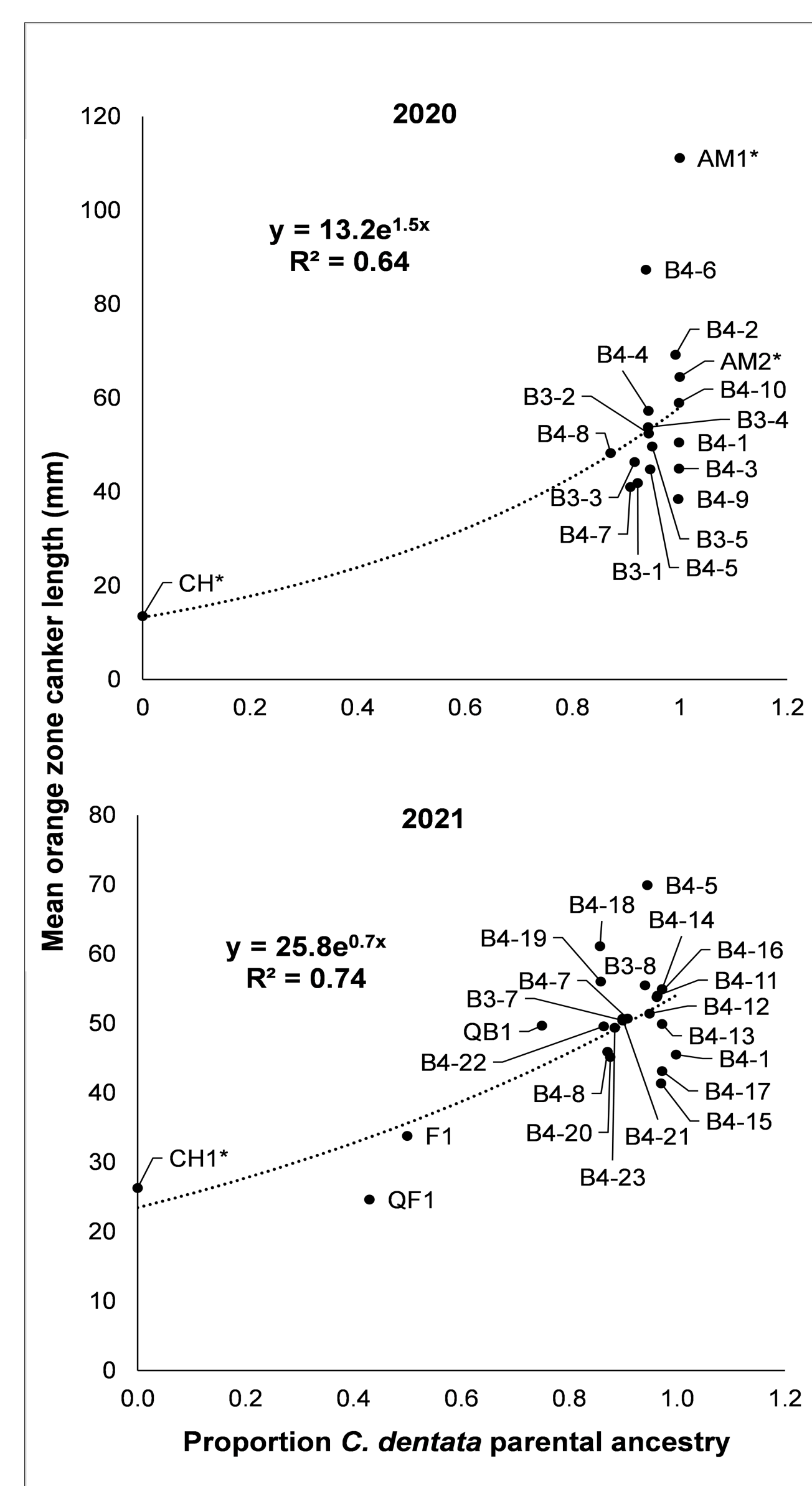
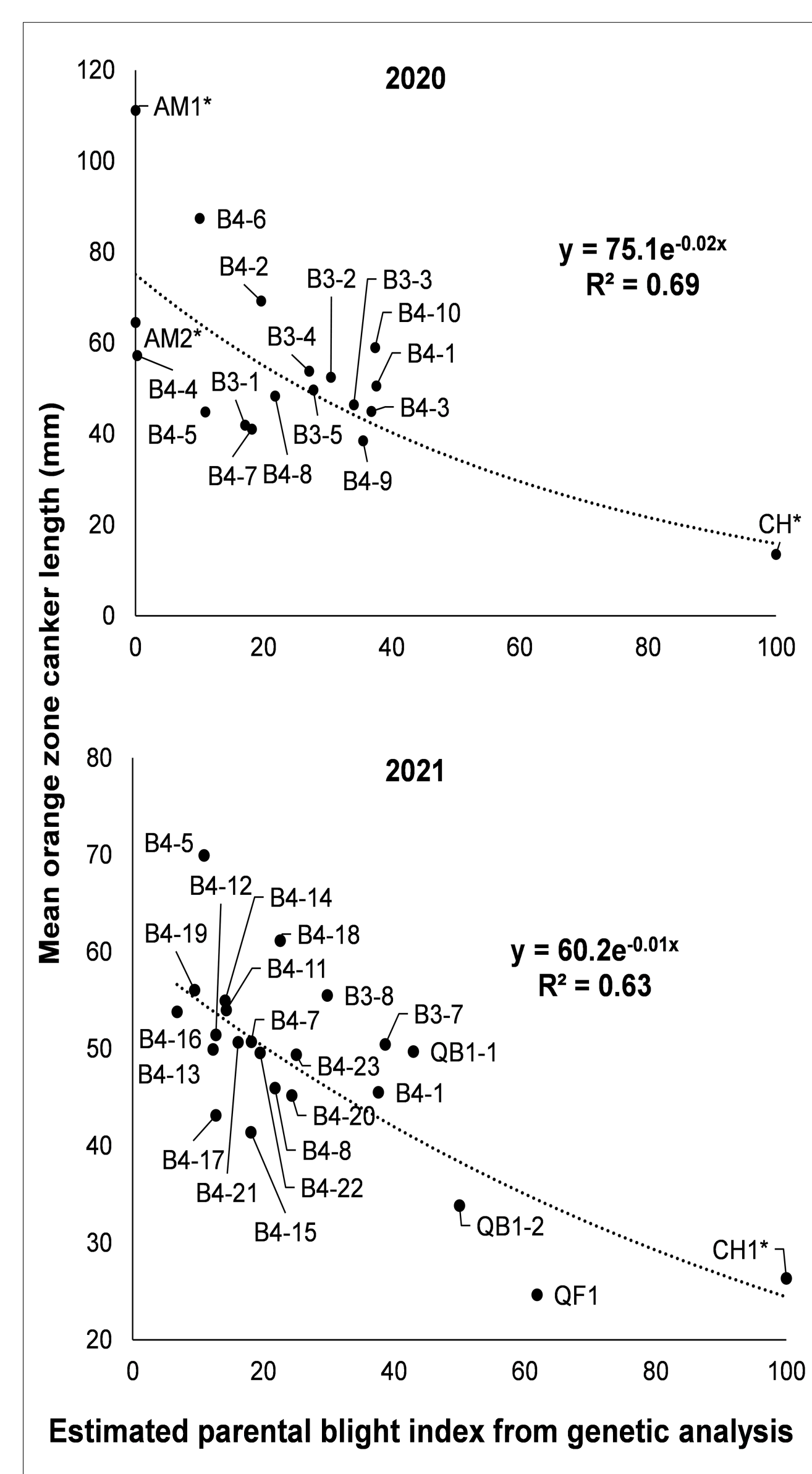
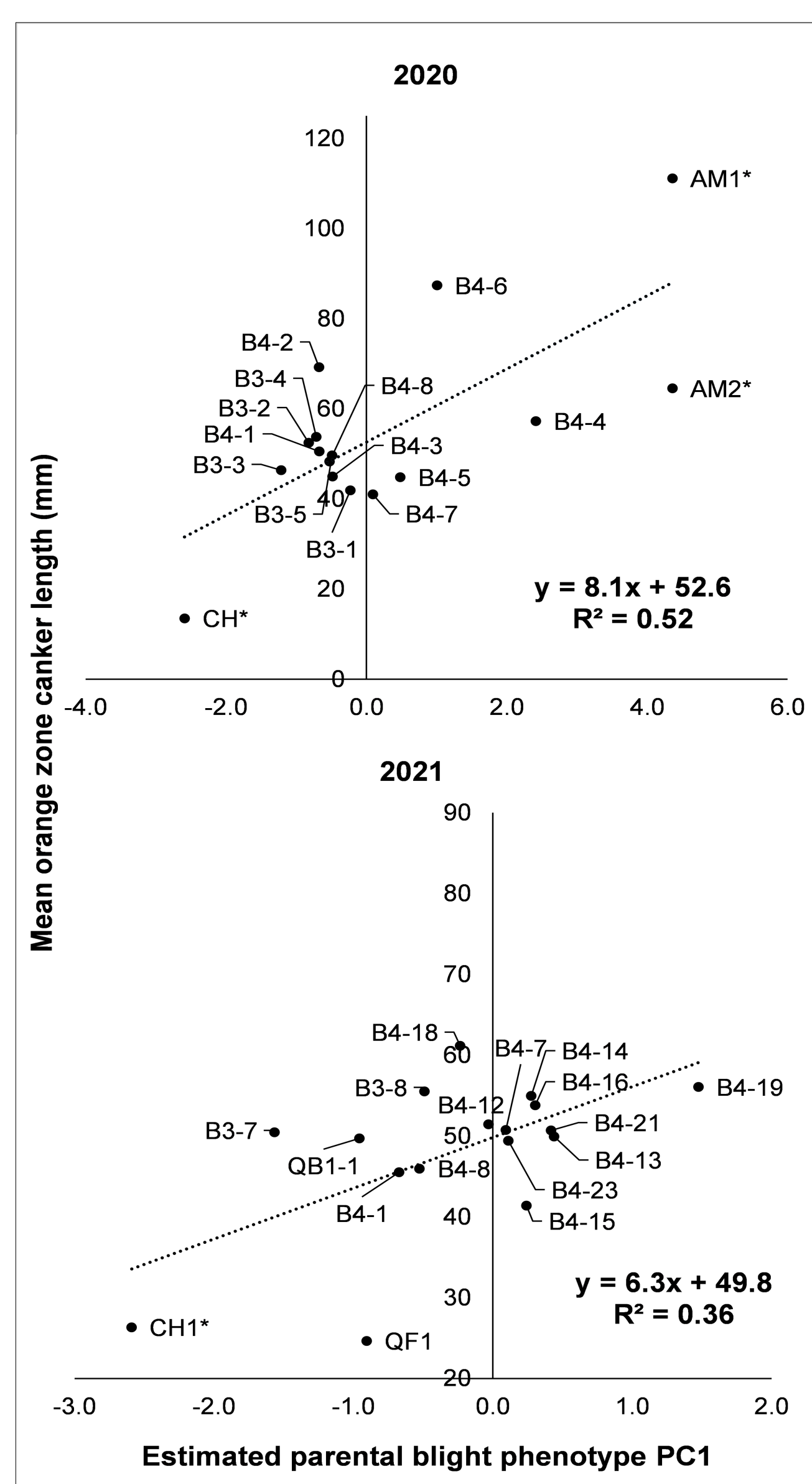


Figure. 6
Status of chestnut seedlings during a follow up visit in May of 2022. Seedlings out planted in February of 2021 (left) and seedlings out-planted in February of 2022 (right) were leafed out and looking healthy. Pre-screening allows for wider spacing and better plant care in the years following planting.

In coming years these seedlings will be further evaluated using traditional stem inoculations followed by genomic analysis of the selected population.

Figure. 3
Seedling OZCL families means regressed on the PC1 index of parental blight phenotype. The PC1 index is scaled such that low values represent resistance and high values represent susceptibility.

Figure. 4
Families means of seedling OZCL regressed on the genetic-based index of blight resistance for parent trees.

Figure. 5
Families means of seedling OZCL regressed on the proportion of AM chestnut ancestry of parent trees.

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Conclusion: A major objective was to ascertain whether the AltSSA can detect the range of blight resistance seen in backcross trees, rather than just the large-scale differences that are observed among pure AM, pure CH and F_1 lines. The spread among mean resistance scores indeed suggests that the AltSSAs can generate results that comport with general expectations, and thus should be good indicators of relative resistance in seedlings. It was also apparent in the relationships of the multi-factor PCA analysis that OZCL alone may be a sufficient index of blight resistance. That said, sporulation had an intermediate loading (i.e. 0.29) on PC1, thus appearing to somewhat relate to severity of infection; so in future studies we plan to better quantify sporulation categories to determine if this character can be more informative.

Implications for AM chestnut restoration: The results suggest that the AltSSA is not only sensitive to resistance differences among backcross lines, but may also enable early forward selection of the most blight resistant progeny within each full sibling family. This discriminative ability may have the potential to accelerate gains made in the TACF breeding programs. Using the AltSSA as a screening method to remove the most blight susceptible trees may also allow for lower initial planting densities, longer survival in the field prior to and/or after natural blight infection, and save time and resources spent growing trees in orchards.