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Leveraging *Populus* Field Trials to Manage Environmental and Human-Induced Change

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Abstract

Tree improvement research is a long-term commitment that is subject to challenges from environmental and human-induced change. Over time, tree health, culture, and economics can be negatively affected by change factors including climate change, pests and/or diseases, regulatory actions, and shifting economic circumstances. In the current paper we describe strategies to leverage **short- and long-term field trials** for managing different types of change that impact tree improvement. Field trials like those presented here could be used to test new materials and processes, as well as serve as an early warning system that measures how various changes may be affecting local and regional tree health. We highlight three main strategies of *Populus* plantation culture for establishing field trials: **1) forest conversion**, **2) low-resource**, and **3) intensive culture hybrid poplar (ICHP) plantation cultures**. In addition, we provide a field trial case study on the low-resource culture type. Replicated *Populus* plantation scenarios could incorporate a mix of native and proven hybrid poplar materials to assess the adaptive behavior of such genotypes across sites with varying soils, climatic conditions, and topography. These field trials could also serve as a long-term archive and clonal/gene pool resource for accelerated tree improvement. While the examples provided in this paper are unique to Michigan, USA, the strategies described are relevant globally for *Populus* and other fast-growing trees grown for environmental and ecosystem services.

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Tree improvement research is a long-term commitment that is subject to challenges from environmental and human-induced change. Over time, tree health, culture, and economics can be negatively affected by change factors including climate change, pests and/or diseases, regulatory actions, and shifting economic circumstances. In the current paper we describe strategies to leverage **short- and long-term field trials** for managing different types of change that impact tree improvement. Field trials like those presented here could be used to test new materials and processes, as well as serve as an early warning system that measures how various changes may be affecting local and regional tree health. We highlight three main strategies of *Populus* plantation culture for establishing field trials: **1) forest conversion**, **2) low-resource**, and **3) intensive culture hybrid poplar (ICHP) plantation cultures.** In addition, we provide a field trial case study on the low-resource culture type. Replicated *Populus* plantation scenarios could incorporate a mix of native and proven hybrid poplar materials to assess the adaptive behavior of such genotypes across sites with varying soils, climatic conditions, and topography. These field trials could also serve as a long-term archive and clonal/gene pool resource for accelerated tree improvement. While the examples provided in this paper are unique to Michigan, USA, the strategies described are relevant globally for *Populus* and other fast-growing trees grown for environmental and ecosystem services.

1. Introduction

Tree improvement projects require substantial investments of time, effort, and land resources (Riemenschneider et al., 2001). Environmental or human-induced changes that occur over the duration of tree improvement projects may distort project objectives and can affect projects well beyond the involvement of the original researchers. Change factors including climate change, outbreaks of pests and diseases, and shifting regulations can all affect the economic viability and long-term sustainability of tree improvement efforts (Chhin, 2010; Stanton et al., 2010). These concerns justify the use of **local** and **regional field trials** for ongoing research that monitors the effects of these changes on tree culture. In this context, **local field trials** refer to relatively long-term field tests that focus on materials, objectives, and cultural practices specific to a local region, state, province, or area (Abrahamson et al., 1990; Coyle et al., 2006; Miller, 2018). **Regional field trials** refer to short- and long-term networks across larger geographic areas that contain replicated clone or yield tests with mixes of the best available materials from forest research centers. Clonal materials in such trials are evaluated for regional adaptability and productivity (Adler et al., 2021; Riemenschneider et al., 2001; Stener and Westin, 2017; Zalesny et al., 2009). A major aim of regional field trials is to better understand genotype × environment interactions within species and clonal mixtures at a regional level in order to identify generalist and specialist genotypes (Zalesny et al., 2021).

Populus field trials are important for proactive mitigation of the change factors noted above that affect tree establishment, research, and livelihoods. Three strategies of *Populus* plantation culture for such field trials are: 1) **forest conversion**, 2) **low-resource**, and 3) **intensive culture hybrid poplar (ICHP) plantations cultures** (Hansen et al., 1984). **Forest conversion** generally involves clear-cutting a forest site, controlling the vegetation for a year, and replanting the site to another species (Zerbe and Kreyer, 2007). Next, a **low-resource process** may be required at sites with constraints that preclude cultural practices such as site fencing, use of tractor equipment, or application of pre-emergent herbicides. Finally, **ICHP** is perhaps the most common method for establishing hybrid poplar plantations, and often involves site preparation activities during the year before planting (e.g., herbicide application, tillage), followed by herbicide applications as needed post planting (Buchman et al., 2020; Lazarus et al. 2015).

2. Monitoring Change Factors

Climate Change

Replicated **regional field trials** can be used to evaluate tree responses to climate change, with the information gathered promoting a better understanding of local forests' responses to shifting climates (Chhin, 2010). Researchers with the USDA Forest Service (USDA FS) are actively studying the impacts of climate change on the nation's forests, with research encompassing climate change adaptation, mitigation, and monitoring (USDA FS, 2022a). For example, the USDA FS' Forest Health Monitoring (FHM) program in the southern Rockies region has used forest health monitoring approaches to assess aspen (*Populus tremuloides* Michx.) forest cover change (Rogers, 2002). This type of historical aspen research may be useful for comparison to native and clonal aspen field trials in similar areas. **Field trials** located on drier upland sites may also provide opportunities for research on species and clonal climate change adaptation strategies (Chhin, 2010; USDA FS, 2022a).

Pests and/or Diseases

Impactful forest pests and diseases can originate from native or non-native sources. Non-native examples include the emerald ash borer (*Agrilus planipennis* Fairmaire) and white pine blister rust (*Cronartium ribicola* J.C. Fisch.) (USDA FS, 2022b). Regarding native pests, the aspen root girdler (*Agrilus horni* Kerremans) has caused substantial damage to plantations of fast-growing trees. In Northern Michigan, for example, the insect has girdled and killed healthy three-year-old *Populus* × *wettsteinii* Hämet-Ahti (*P. tremuloides* × *P. tremula* L.) on Lyme Timber Company aspen forest conversion plantations grown near native aspen stands that host indigenous populations of the insect (Lyme Timber Company, n.d.; Steed and Burton, 2015; USDA FS, 2022c). Monitoring **local** and **regional field trials** for pest and disease outbreaks may play a vital role in early detection and understanding of these issues.

Regulations and Economic Feasibility

Federal, state, and corporate landowners may leverage laws, regulations, and certifications to help manage social, environmental, and economic forest sustainability. For example, wood products derived from certified managed forests provide a market-based approach to recognize sustainable forest practices (Fernholz et al., 2021; FSC, 2015; SFI, 2015). Regulations can also present challenges for forest managers by setting eligible stock types (native vs non-native), labor restrictions, and plantation culture (e.g., whether to use herbicides) (USDA FS, 2012). These external constraints raise the importance of using **tree improvement field trials** to test and deliver more robust materials and productive plantings.

3. *Populus* Field Trials for Managing Change

Two *Populus* field trial case studies are presented. The first case study, summarized in **Box 1**, highlights an assessment of *A. horni* management within a *Populus* planting located near La Branche, Michigan, USA. The second case study, which is the focus of the remainder of the paper, is a review of a **long-term, low resource, replicated field trial** of native aspen and hybrid poplar materials at a highway right-of-way site located near Belmont, Michigan, USA. A hypothetical scenario for expanding the extent of the plantings at this site is provided in the final section. While these examples include *Populus* genotypes grown in Michigan, USA, the strategies described are relevant globally for *Populus* and other fast-growing trees grown for environmental and ecosystem services.

Box 1

Case Study 1: Lyme Timber Company Aspen Root Girdler (*Agrilus horni* Kerremans) Field Trial

Background

Aspen **forest conversion plantings** of *Populus* × *wettsteinii* were established by the Lyme Timber Company at a site near La Branche, Michigan, USA (45.8775 °N, -87.4690 °W) (Figure 1). Trees were established on 10 May 2017 at a stocking density of 170 trees per hectare. Trees at the site exhibited infestations with the aspen root girdler, *Agrilus horni* Kerremans (Figure 2). In 2018, at the beginning of the second growing season, the Lyme Timber Company established a 600-tree field trial in these plantings to evaluate the effects of Coretect^{TM} insecticide tablets (Bayer Environmental Science, Cary, North Carolina, USA) on tree survival. Two, 300-tree blocks were established to test two types of insecticide tablet applications (Riley, 2022). In each block, 150 trees were treated, and 150 trees were not treated. In one block, treated trees received surface applications of the insecticide tablets (i.e., one tablet was placed on the ground at the base of each tree). In the other block, treated trees received belowground applications of the tablets (i.e., one tablet was buried 1-2 in below the soil surface at the base of each tree). Within both blocks, alternating live trees either received one tablet or received no tablet.

Results

The *A. horni* field trial concluded at the start of the fifth growing season (2021). Within the surface application block, the survival rate for treated trees was 98.7%, compared to 68.7% for non-treated trees. For the belowground application block, survival rates were 99.3% and 76.6% for treated and non-treated trees, respectively. The Coretect^{TM} treatment was highly effective at the beginning of the second growing season, with no substantial differences observed between the surface applications and the belowground applications (Patrick Riley, personal communication). Due to higher survival rates, stocking density could be reduced from 170 to 100 trees per hectare, and Coretect^{TM} treatments could be applied at the beginning of the second and fifth growing seasons, with potential coppicing occurring approximately ten years after planting in order to repopulate the site (Riley, 2022).

Box 1 *(cont'd)*

Figure 1. Location of *Populus* × *wettsteinii* plantings established by the Lyme Timber Company at a site near La Branche, Michigan, USA.

Figure 2. Damage on *Populus* × *wettsteinii* from aspen root girdler, *Agrilus horni* Kerremans, at a Lyme Timber Company forest conversion planting near La Branche, Michigan, USA.

Case Study 2: Open4st Low-Resource Local Aspen Field Trials

Open4st Plantings: Background

The Open4st group has developed breeding and selection strategies for purpose-grown hybrid aspens since 1985 (Figure 3) (Open4st, 2010). These breeding efforts have produced 234 families and tracked 329 clones, with test materials planted at six non-replicated sites in southwest Michigan, USA, and replicated locations in Iowa, Michigan, Kentucky, Indiana, Minnesota, and Wisconsin, USA. Michigan State University's Forest Biomass Innovation Center (FBIC) conducted a *Populus alba* L. progeny test in 2005 and a clonal trial in 2010. The 83xAA04 *P. alba* family was found to grow well, resist disease, propagate readily from dormant stem cuttings, and have ortets with variable growth rates (Miller et al., 2017). Other Open4st *Populus* projects include the r4st plant database, aspen propagation from seed and rootlings, active shoot propagation (ASP), and the layered aspen whip (LAW) process (McGovern and Bender, 2018). Additionally, Open4st developed a *Salix* cultivar database for Working Party 1 (Taxonomy, Nomenclature, and Registration) of the International Commission on Poplars and Other Fast-Growing Trees Sustaining People and the Environment (IPC) of the Food and Agriculture Organization of the United Nations (UN FAO) (McGovern et al., 2021).

Figure 3. Aspen seedlings produced by Open4st breeding and selection strategies.

Open4st Plantings: Naming Conventions

Most Open4st planting sites are located along US Highway 131 (US 131) in the lower peninsula of Michigan, USA. Open4st southwest Michigan sites planted before 2018 consist of unreplicated archival plantings located on unfenced Michigan highway right-of-way property that is restricted by an annual permit approval process. These Open4st plantings are named to provide information on both the name of the nearest exit ramp highway and the direction of the nearest interchange. For example, the site involved in this case study, **PostSE**, gets its name from the US 131 **Post** Drive exit ramp and the **SE** interchange, which are located adjacent to the planting.

Site Description: PostSE Case Study Site

Since 1988, Open4st aspens have been planted near Belmont, Michigan, USA (43.0874 °N, -85.6317 °W) at a site adjacent to the Post Drive SE highway interchange (Figure 4, Figure 5). This site, known as PostSE, consists of nearly 2.3 hectares that were planted with 451 trees between 1988 and 2006 (Table 1). These trees were typically planted at densities of 2.4 × 2.4 m. However, 133 trees in 2005 and 18 trees in 2006 were planted at 1.2 × 2.4 m spacing.

The entire PostSE site is classified as Spinks loamy sand (30C) with six to twelve percent slopes (USDA NRCS, 2021). Despite the site's well-drained drainage class (USDA NRCS, 2021), a west-to-east gradient of higher to lower soil fertility and plant growth has been observed across the entire site (Table 2). The southeastern half of the PostSE site is excessively drained, with many areas having exposed sand with lichen and stubble grass that wilts during drought. In these areas it is difficult to plant trees with a dibble bar due to the hardpan and gravel.

The information presented in the current case study pertains to aspen materials planted after 2018 at the PostSE site, with a common planting density of 2.4 × 2.4 m and one or more border rows spaced at 1.4 × 2.4 m for stoolbed cutting production (i.e., coppicing of aspen trees to produce dormant hardwood cuttings for subsequent planting). Three plantings were established at the site: **PostSE1**, **PostSE2**, and **PostSE4.1** (Figure 6). Trees were established at the PostSE site with the objectives of 1) comparing variability in adaptation to site and climate conditions, and 2) testing for differences among genotypes to identify superior families and clones.

Figure 4. Location of Open4st PostSE *Populus* plantings established near Belmont, Michigan, USA.

Figure 5. Open4st PostSE field planting near Belmont, Michigan, USA.

Table 1. Open4st PostSE site planting descriptions, including the species and number of trees planted each year.

Pre-2018 PostSE Site Planting Description		
Year Planted	**Number of Trees Planted**	**Species, Genomic Groups, Clones**
1988	30	*Populus alba* L. *P. alba* × *P. grandidentata* Michx. 'Crandon' *P. deltoides* Bartr. ex Marsh × *P. nigra* L. 'DN34'
1989	18	'Crandon' 'DN34' Family 1xAGA88 (*P.* × *rouleauiana* × *P. alba*)
1994	26	*P.* × *smithii* B. Boivin families (×3) *Quercus robur* L.
1996	51	*Q. robur* *P. tremuloides* Michx. *P. alba*
2004	6	Family 79xAA03 (*P. alba* × *P. alba*)
2005	273	*P. alba* families (×6) T×E Triploids: Family *Populus* × *wettsteinii* Hämet-Ahti (*P. tremuloides* × *P. tremula* L.) *P. deltoides* × *P. nigra* L. 'DNC0018' 'DN34' *P. nigra* × *P. maximowiczii* A. Henry 'NM6'
2006	47	Family 83xAA04 (*P. alba* × *P. alba*) Family 1xA4E05 (triploid): *P.* × *canescens* (Aiton) Smith 'DN34' 'NM6'
Total	451	

Table 2. 2021 height growth and survival of aspen grown at the Open4st PostSE4.1 site.

PostSE4.1 Block Summary - 2021				
Block	**Mean Height (cm)**	**Number of Trees Planted**	**Number of Live Trees**	**Mean Survival Rate (%)**
1	141.3	16	16	100
2	107.5	16	16	100
3	83.1	16	14	87
4	108.8	16	16	100

Figure 6. Planting layout of three Open4st *Populus* plantings (PostSE1, PostSE2, and PostSE4.1) established near Belmont, Michigan, USA

Experimental Design

The PostSE field trials planted from 2019 to 2022 were typically replicated with 2- or 4-tree plots within multiple blocks, depending on the planting year, treatment, and spacing. The **PostSE1** and **PostSE2** plantings were established in 2019, 2020, 2021, and 2022, while the **PostSE4.1** planting was established in 2021. For **PostSE1**, a total of 264 *P. alba* hybrid trees were planted at 2.4 × 2.4 m spacing across five experimental blocks. Two southern rows were established at 1.2 × 2.4 m spacing for use as future stoolbeds. For **PostSE2**, a total of 67 trees were planted at 2.4 × 2.4 m spacing across four experimental blocks, with 43 trees being progeny or clones of *P. grandidentata*. The northernmost row was established at 1.2 × 2.4 m spacing for use as future stoolbeds. Fencing was not used for any of the PostSE plantings, and therefore deer browse was an important factor to consider when establishing these plantings. As native aspen usually exhibits slower growth and greater difficulty of establishment under high deer pressure when compared to *P. alba* materials (Li et al., 1993; Myking et al., 2011), the **PostSE2** planting was established south of **PostSE1** to prevent shading bias from the faster-growing hybrids. For **PostSE4.1**, a total of 96 trees were planted at 2.4 × 2.4 m spacing across four experimental blocks. Each of the four blocks was composed of two rows with four-tree plots and five treatments for a total of 64 trees. An additional southern row was planted with 32 dormant cuttings of 36-cm length (control clone '8bg3').

Management Operations

The **low-resource**, no-till site preparation consisted of planting dormant, hybrid aspen hardwood cuttings 36 to 46 cm in length or bare root “ministools” having an 8-cm root ball on 2.4 × 2.4 m centers with control clones (Figure 7). In 2021, each tree planting area was first aerated with a garden fork (Talbot, 2021), and the stock was then planted 25.4 cm deep with a Jim-Gem® KBC planting bar. Stems were protected from deer, rabbits, and voles by 46-cm or 91-cm fabric sleeves stapled to 1.2-m bamboo stakes. Trees planted in the PostSE4.1 site were all protected by 46-cm tree shelters, while in the 2020 PostSE1 planting, every other tree had a short 46-cm or tall 91-cm tree shelter. Glyphosate was sprayed to control vegetation in 1.2-m mid-row strips several times during the first two growing seasons. All trees planted in 2021 were pruned to a height of 60.96 cm during July 2021 to promote multi-branched stems that discourage deer rubbing and protect against over-browsing (McGovern, 2022), and to encourage consistent stem branching across all PostSE plantings.

Figure 7. Bare root aspen “ministools” planted at the Open4st PostSE plantings near Belmont, MI, USA.

Data Collection

Tree height was measured at all plantings, but diameter was not measured for all trees due to growth setbacks from deer damage. Trees were binary scored (Yes or No) in December 2021 for any new rubbing damage after the peak deer rubbing activity (Figure 8). Trees were also scored for overall leaf health and for bronze leaf disease (Figure 9). Table 3 describes a five-point scoring system that was used for ranking both generic and BLD leaf issues, which consists of a subjective assessment of how much of the crown is affected. A leaf score of 1 indicates that over 50% of the crown is affected by leaf issues. Conversely, a score of 5 indicates healthy, robust, and clean leaves. Average leaf and BLD scores, therefore, reflect observed leaf issues and defoliation. Data on all the recent PostSE plantings, trees, and measurements are recorded in the Open4st online r4st database (available at https://tinyurl.com/r4stdatabase).

Figure 8. Severe deer rubbing on aspen at the Open4st PostSE site near Belmont, MI, USA.

Figure 9. Leaf affected by bronze leaf disease (BLD) at the Open4st PostSE site near Belmont, MI, USA.

Table 3. Generic and Bronze Leaf Disease (BLD) leaf scoring criteria.

Open4st Leaf Scoring Criteria	
Score	**Description**
1	Over 50% of crown affected by leaf issues, major defoliation.
2	At least 25% of crown affected by leaf issues and defoliation.
3	Leaves moderately affected with minor defoliation.
4	A few leaf issues, no defoliation.
5	No leaf issues.

Results and Discussion

Open4st 2021 PostSE Site Results

Survival, height, leaf issues, and deer impacts for all three PostSE plantings are listed in Table 4. The more fertile western end of the PostSE1 planting had the largest three-year-old single-plot tree ('20bs8'), which had a height of 700 cm in 2021. Nearby clones of '11ab10' and '20bs3' each had heights of 600 cm. The standard deviation of 147.38 for PostSE1 shows considerable west-to-east height variation and provided the rationale for using four west-to-east blocks with four-tree square plots for the PostSE4.1 planting.

Open4st 2021 PostSE Deer Rubbing Results

Table 4 lists deer rubbing metrics measured in 2021 for all three PostSE plantings. The one-year-old PostSE4.1 planting had 17% of the stems rubbed, perhaps due to existing travel corridors near bedding cover and a preference for deer to rub smaller trees in that area. For planting PostSE1, ten of the rubbed trees were planted on two-tree plots with either 46-cm (six trees) or 91-cm tree shelters (four trees).

Table 4. Survival, buck rub, growth, and leaf scores measured in 2021 at site PostSE.

Site Name	Number of Trees Planted	Mean Survival (%)	Trees Rubbed (%)	Height[a] (cm)	Mean Leaf Score	BLD Leaf Score
PostSE1	256	96	6	290.2 ± 147.4	4.11	4.66 ± 0.83
PostSE2	62	73	3	121.2 ± 105.0	3.98	4.92 ± 0.24
PostSE4.1	96	91	18	101.5 ± 32.6	4.07	5.00 ± 0.00

[a] Mean ± one standard deviation

Open4st 2021 PostSE BLD Leaf Issue Results

Results of the 2021 BLD leaf scoring for 62, three-year-old hybrid aspens at the PostSE1 planting are presented in Table 5. These PostSE plantings may provide a desirable environment for leaf disease infection due the historical existence of adjacent aspen stands having known BLD issues. Sixty percent of the aspens had a score of 5 (i.e., healthy, robust, clean leaves) with no observable BLD, three percent had a score of 1, indicating major leaf defoliation, and 86% of the aspens had little or no BLD at age three. It will be useful to track infection and survival rates with clonal, family and site interactions over time.

Table 5. Average BLD scores for three-year-old trees at site PostSE1.

BLD Leaf Score	Number of Trees	Percent
1	2	3%
2	4	6%
3	3	5%
4	16	26%
5	37	60%
Sum	**62**	**100%**

Table 6 lists average leaf and BLD scores and DBH values for three-year-old trees of clones at the PostSE1 planting with at least two surviving individuals (i.e., live quantity > 1). The widely tested clone 'DN34' [(*P. deltoides* Bartr. ex Marsh. × *P. nigra* L. (a.k.a., *P. × euramericana* cv. 'Eugenei')] (Hansen et al., 1993) was used as a control clone on Open4st sites and is not susceptible to BLD; therefore, it was not scored for this parameter. However, 'DN34' consistently had a leaf score of three due to poplar leaf rust (*Melampsora medusae* Thüm.) and defoliation issues. The generic leaf scores are somewhat independent of BLD scores since a tree could score 5 for BLD but have an overall score of 4 for generic leaf issues. Four *P. alba* hybrid clones consistently scored 5 for BLD with zero population standard deviation. Likewise, there were five *P. alba* hybrid clones with variable BLD scores that had standard deviations greater than 0.40. Clone ‘25r10‘ is the only F_1 cross among these PostSE1 *P. alba* hybrid clones. The other *P. alba* crosses are F_2 backcrosses with '8bg3', '23ba2', '23ba15', '23ba18', '22bg1', '1bw6', and '20bs3' sharing the same female parent ('cag204'). It is also interesting to note that the three 23ba named clones are siblings with clones '23ba2' and '23ba15', both of which scored 5 for BLD.

Initially, it was expected that the *P. alba* F_2 backcrosses would exhibit the greatest variation and selection challenges due to recombination of various traits. However, the F_1 25xr family having the figured male parent 'GG12' has shown considerable variation for figure, as seven of eighty-two clones (8.5%) showed medium to high figure and a chlorotic leaf condition that varies per clone but was easily selected in the first-year nursery stage (Open4st, 2019). Eighteen of these clones (60%) belong to the 25xr family and had a leaf score of 5 with no observable leaf issues (Open4st, 2019).

Table 6. Diameter at breast height (DBH), general overall leaf scores, and bronze leaf disease (BLD) scores of three-year-old trees at the PostSE1 planting. The PostSE1 planting is located adjacent to an aspen stand that exhibited substantial long-term BLD and associated mortality.

Clone ID	Live Qty (>1)	DBH Rank	Mean DBH (cm)	Mean Overall Leaf Score	Mean BLD Leaf Score[a]
23ba18	3	1	6.06	4.33	4.33 ± 0.47
11ab10	3	2	5.26	2.66	4.66 ± 0.47
19gb21	2	3	5.05	4.00	4.00 ± 0.00
25r10	3	4	5.00	2.66	2.33 ± 0.47
1bw6	14	5	4.72	4.21	5.00 ± 0.00
23ba15	4	6	4.20	4.50	5.00 ± 0.00
DN34	10	7	4.03	3.00	N/A
20bs3	2	8	3.80	4.50	5.00 ± 0.00
23ba2	2	9	2.95	4.00	5.00 ± 0.00
22bg1	8	10	2.48	4.12	4.62 ± 0.48
8bg3	9	11	2.47	4.11	4.22 ± 0.42
Totals:	**60**		**4.18**	**3.83**	**4.42**

[a] Mean ± one standard deviation

Table 7 compares height and BLD scores for the highly susceptible clone '25r10'. All three '25r10' trees had BLD at ages two and three, suggesting that it could be used as a control clone for early BLD detection on some field trial sites.

Table 7. Second- and third-year height and BLD scores of clone '25r10' at the PostSE1 planting. Clone '25r10' is highly susceptible to BLD.

Tree Number	2020 Height (cm) (age 2 years)	2020 BLD Leaf Score (age 2 years)	2021 Height (cm) (age 3 years)	2021 BLD Leaf Score (age 3 years)
1	350	4	600	3
2	280	4	450	2
3	243	3	330	2
Average:	**291**	**3.67**	**460**	**2.33**

The outplanting conducted above depended upon Open4st nursery production of cutting material. Average survival for Open4st clones grown from 20-cm dormant cuttings was greater than 92% for all clones (Table 8). Clones were tested as they became available; therefore, there was an uneven distribution in the number of cuttings tested per clone, with cutting numbers distorting clonal survival rates. Control clone 'DN34' was propagated over eight years with 405 stems and an average cutting diameter of 7.7 mm. The 2019 propagation results include sixteen 'DN34' cuttings that had an average diameter of 12 mm and 100% survival for comparison purposes. It is interesting to note the 93% survival rate of the F_2 '1bw6' backcross clone over 4 years, the 100% survival rates of the four 25xr F_1 clones, and that the 15 clones included in Table 8 are derived from 8 different families. Perhaps an important program theme could incorporate genetic diversity while increasing propagation traits to address new requirements for both environmental and human-induced change.

Table 8. Average survival rates of Open4st nursery dormant cuttings.

Clone ID	Number of Nursery Testing Years	Number of 20-cm Dormant Cuttings	Filial	Mean Survival Rate (%)	Mean Cutting Diameter (mm)
1bw6	4	97	F_3	93	11.41
3bc4	2	14	F_3	93	11.66
14b10	3	30	F_3	95	9.25
14b40	2	11	F_3	95	13.00
14b41	2	11	F_3	95	11.50
105aa3	2	34	F_1	96	9.20
105aa5	2	34	F_1	97	10.60
20bs1	3	25	F_3	97	11.66
25r170	2	14	F_1	100	13.50
25r12	2	9	F_1	100	11.50
107aa7	2	9	F_3	100	11.50
20bs5	2	9	F_3	100	11.00
25r7	2	9	F_1	100	10.50
25r1	2	9	F_1	100	14.00
23ba4	2	9	F_3	100	9.00
DN34 (Control)	8	405	F_2	88	7.70
DN34 (2019)	1	16	F_2	100	12.00
Average Totals:	**3**	**44**		**97**	**11.12**

Prominent PostSE Change Factors

The prominent change factors affecting PostSE plantings are: 1) climate change, 2) tree pests and/or diseases [specifically, bronze leaf disease (BLD), a persistent issue for susceptible aspens that is caused by the fungal pathogen *Apioplagiostoma populi* Barr (Smith et al., 2002)], and 3) impacts from wildlife populations. The Open4st PostSE plantings can be leveraged to mitigate these issues by enabling: 1) selection of productive clones for upland sites, 2) identification of clones resistant to tree diseases, and 3) testing and development of cost-effective tree shelters.

Selecting Productive Upland Site Clones

While fertile, well-drained sites are preferred, they are in limited supply, and planting on drier upland sites may present selection opportunities for testing clones adaptable to a changing climate. The upland PostSE4.1 planting, with its southerly exposure, could be useful for planting and selecting clones suitable for a drier climate using **low-resource processes**.

Selecting Clones Resistant to Bronze Leaf Disease (BLD)

Several *P.* × *rouleauiana* (*P. alba* × *P. grandidentata*) and other *P. alba* hybrid clones have been observed with a high degree of annual BLD infection. Likewise, some PostSE clones exhibit low or no BLD issues, suggesting clonal variability in BLD resistance. Other *P. alba* hybrid backcrosses, such as '8BG3' and '8BG9', exhibited BLD, but to a lesser degree and with later infection rates. Clones '25r30', '25r31', and '25r10' were heavily infected with BLD in both 2020 and 2021. Other nearby Open4st sites with *P. alba* hybrids (planted since 1992) have surviving families with clones showing variable degrees of BLD resistance. A wild *P.* × *rouleauiana* clone ('AGRR1') (Open4st, n.d.) was selected several kilometers from the PostSE site in 1991. The clone was estimated to originate in 1960 and was established on other Open4st sites. While this clone often shows annual BLD infection on older stems, it performs well and sprouted vigorously on the original site after straight-line winds felled trees in May 2014. Planting field trials near established plantings can help detect and select clones for disease issues earlier than with new sites.

Developing Cost-Effective Tree Shelters

The PostSE site has sustained viable deer, rabbit, and vole populations over time. Deer rubs and browsing may inhibit growth and cause stem damage to young aspen stands (Myking et al., 2011). Stem breakage occurring on healthy *P. alba* materials usually results in vigorous resprouting on the stem, in addition to root suckering. However, repeated rubbing and browsing of young native aspen saplings can affect growth and survival. In general, damage from deer browse and rubbing occurs on site edges or game trails, with trees being able to recover from occasional damage. We have used both 46- and 91-cm fabric and bamboo-staked tree shelters in replicated two-tree plot trials containing both sizes at the PostSE plantings. General observations suggest that the 46-cm shelters encourage shorter and branchier stems that are less likely to be over-browsed or buck rubbed, while trees protected by 91-cm shelters may have smaller diameter stems that can break at ground level when severely rubbed (Figure 10). The tree shelters implemented at the PostSE plantings were assembled by McGovern and cost about $0.50 USD each. Other 46-cm tree plastic shelters are available commercially, albeit more expensive (McGovern, 2022). *P. alba* hybrid saplings tend to survive deer rubbing or other damage with robust re-sprouting if stem damage occurs after August. On the other hand, native aspens grown on PostSE plantings are less vigorous, grow slower, and often do not survive repeated damage from deer browse and rubbing.

Figure 10. Fabric deer shelters of two different heights used on aspens at the Open4st PostSE site near Belmont, MI, USA. The 46-cm shelters encouraged shorter, branchier stems (tree in bottom right of photo), while the 91-cm shelters promoted smaller diameter stems with less branching (tree on far-left side of photo).

4. Hypothetical Open4st Long Term Field Trial Strategy

A 96-tree field trial was planted at the PostSE4.1 site in 2021. The entire PostSE4 site has an approximate capacity of 1,000 total trees planted at 2.4 × 2.4 m spacing (34 rows × 30 columns). This southerly-exposed site could prove useful for selecting clones suitable for a drier climate using **low-resource processes**. A **hypothetical long-term PostSE4 field trial strategy** could involve the following events, in chronological order:

Plant PostSE4.2 Site

- Conduct a soil test for each block prior to planting. Plant 384 trees using 12 clones on 2.4 × 2.4 m centers with four blocks and each block having two, four-tree randomized square plots (96 trees per block with two plots per clone and a planting total of 32 trees per clone). The planting could be established using 12 east-west rows with 32 north-south columns.
- Prepare the four-tree plots by aerating each tree location with a garden fork, adding one cup of a soil amendment treatment to two of the four randomized plot holes, then planting the 45.72-cm ministools.
- Apply site management best practices:
    - 46-cm fabric tree shelters
    - Two seasons of no-till
    - 1.2 m strip applications of glyphosate
    - Stem pruning to 60 cm during mid-summer of the first year
- Possible planting stock:
    - Open4st aspens and external materials such as the 'DN34' control clone, *Populus canescens* clone ‘717’ (*P. tremula* × *P. alba*) and *P.* × *wettsteinii* (*P. tremuloides* × *P. tremula*).
- A single border row could be planted around the trial with a control clone such as 'DN34' planted alternately as 45.72-cm dormant cuttings or 1-0 bare root stock.
- Native aspens typically grow slower than the hybrid selections, requiring separation and more southern light exposure. Therefore, two southern rows could be planted with four-tree plots having native aspen seedlings or bare root trees represented from local and distant aspen populations.
- The southernmost row could be planted at 2.4-m spacings with a mix of clones used in the trial. They would serve to archive stock by pollarding them at 1.2-m height at regular intervals for on-demand cutting production.
- Trees that did not survive the first year could be replanted the following year and recorded in a site database.

- Future trial plantings could extend south of the existing materials and use the same spacings to reduce shading effects.

Measure PostSE4.2

- Measure survival and height after the first growing season and diameter every third year thereafter. Note substantial disease, insect, and animal damage. Store data in the Open4st online r4st database (McGovern, 2020) and post online with a summary and relevant observations. Over time, develop an accelerated tree improvement strategy that would also consider any external change concerns.

Plant PostSE4.3

- Every six years, review the data and consider a new field trial planting to re-test existing clones and/or breed new materials based on past results. Select a mix of the top performers to capture those aspen materials that perform better in later years.
- Repeat the above process as resources allow.

5. Conclusions

Localized field trials could involve long term resources to implement, monitor and maintain accelerated tree improvement programs. Therefore, ongoing **long-term local field trials** could be integrated with tree improvement centers using their existing cultural and technical processes. Additionally, **short-term regional field trials** could be planted on a regular basis with a mix of materials from each of the tree improvement centers. They could be planted across multiple states using **ICHP processes** similar to the *Populus* biomass regional field-testing network in the midwestern United States (Riemenschneider et al., 2001; Zalesny et al., 2009). **Integrated short- and long-term field trial strategies** may help researchers monitor, test, and deliver sustainable options to mitigate the impacts of environmental and human-induced change.

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