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Selecting *Populus* with different adventitious root types for environmental benefits, fiber, and energy

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Abstract

Primary roots from seeds, sucker roots in aspens, and adventitious roots (ARs) in poplars and their hybrids are prevalent within the genus Populus. *Two AR types develop on hardwood cuttings: (i) lateral roots from either preformed or induced primordia along the length of the cutting and (ii) basal roots from callus at the base of the cutting in response to wounding imposed by processing the parent shoot into propagules. Adventitious rooting is a key trait in*

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Populus *tree improvement programs because of the need for inexpensive plantation establishment with genotypes that perform well across heterogeneous environments or are better adapted to local site conditions. The objective of this chapter is to describe how selecting* Populus *with ability to develop both AR types can be used to provide environmental services necessary for long-term ecosystem sustainability across multiple temporal and spatial scales, including as carbon (C) sequestration and environmental remediation, fiber for the paper industry, and energy feedstocks such as cellulosics for ethanol and biomass for electricity. The anatomical origins (anatomy), accumulation and partitioning of C and carbohydrates (physiology), quantitative and molecular genetics of rooting, and propagation and site conditions that affect root initiation and growth (external factors) are described to provide background information about the development of ARs from initiation to application. The usefulness of adventitious rooting for environmental sustainability is detailed, including the importance of combining the selection of unique genotypes with lateral, basal, or some level of both AR phenotypes to optimize the productivity of the short rotation* Populus *crops for environmental benefits, fiber, and energy.*

Introduction

Populus tree improvement programs in the United States began in the 1920's (Northeast) and 1930's (Midwest) with twelve species consistently tested as parents in the crosses [1]. Since that time, more than 100,000 unique genotypes have been developed in the Midwest [2,3]. *Populus* species and hybrids are ideal for genetic improvement because of their ease of propagation, relatively short generation time, and broad range of genetic variation [4-6]. Most of the genetic diversity of *Populus* is at the genus level [7], which aids successful intra- and inter-sectional hybridization within and between most of the six taxonomic sections, especially *Aigeiros* and *Tacamahaca* [8,9]. Fifty-six percent of elite parents from the original *Populus* tree improvement programs were from these sections (29% *Aigeiros*; 27% *Tacamahaca*) [1], while parents from these sections constitute nearly 100% of recent breeding efforts [10-12]. Four species are most commonly used in North American *Populus* breeding: *P. deltoides*, *P. trichocarpa*, *P. nigra*, and *P. suaveolens* subsp. *maximowiczii*. There are substantial differences among these species for economically and ecologically important traits, including adventitious root (AR) formation, yield, and insect and disease resistance (Table 1).

There is broad genetic variation in adventitious rooting of *Populus* [13-15], which provides the potential for substantial realized selection gains.

Table 1. Variation of important traits among four *Populus* species most commonly used for environmental benefits, fiber, and energy in the midwestern United States.

Species	Rooting Ability[z]	Potential yield	Insect resistance	Disease resistance
P. deltoides	Erratic	Very good	Poor	Good
P. trichocarpa	Very good	Good	Good	Very bad
P. nigra	Good	Good	Very good	Poor
P. suaveolens* subsp. *maximowiczii	Very good	Good	Good	Poor

[z]Adventitious rooting from dormant hardwood cuttings.

As a result, breeding for enhanced rooting ability has always been a key component of *Populus* clonal development [1] and continues to gain support in current programs [16,17].

Understanding genetic and physiological mechanisms promoting or hindering rooting enhances the potential for successful commercial deployment because rooting is the first biological requirement for stand establishment [17-19]. *Populus* trees are often planted as unrooted dormant hardwood cuttings and success depends upon the initiation and development of ARs [20]. There are two distinct AR types from dormant cuttings [21-23] (Fig. 1). The first type is *lateral root development* from latent preformed primordia distributed throughout the length of the cutting [24] or from induced primordia as a response to external stimuli [21]. The second type is *basal root development* differentiated from callus at the base of the cutting [17]. Callus development, a common wound response in forest trees and horticultural woody plants, results from processing the parent shoots into the cuttings.

Using *Populus* for environmental benefits, fiber, and energy depends upon successful rooting and subsequent establishment. The four species listed above and their hybrids grow approximately 6 to 8 times faster than native aspen (*P. tremuloides* and *P. grandidentata*) in the midwestern United States, with aboveground stand productivity between 27 to 45 m^3 ha^{-1} yr^{-1} compared with 4 to 6 m^3 ha^{-1} yr^{-1} for *P. tremuloides* and *P. grandidentata* [10,25]. In addition, there is a projected shortage of native aspen in suitable diameter classes within the next decade [26], which threatens fiber supply. The use of *Populus* for remediation phytotechnologies [2,27,28], riparian buffer systems [29,30], and carbon sequestration [31-33] has become increasingly important as worldwide ecological degradation increases. Furthermore, increased fossil fuel prices have made short rotation *Populus* crops more economically viable than in previous years for the production of biofuels, bioenergy, and bioproducts [34,35]. Record oil, natural gas, and transportation fuel prices

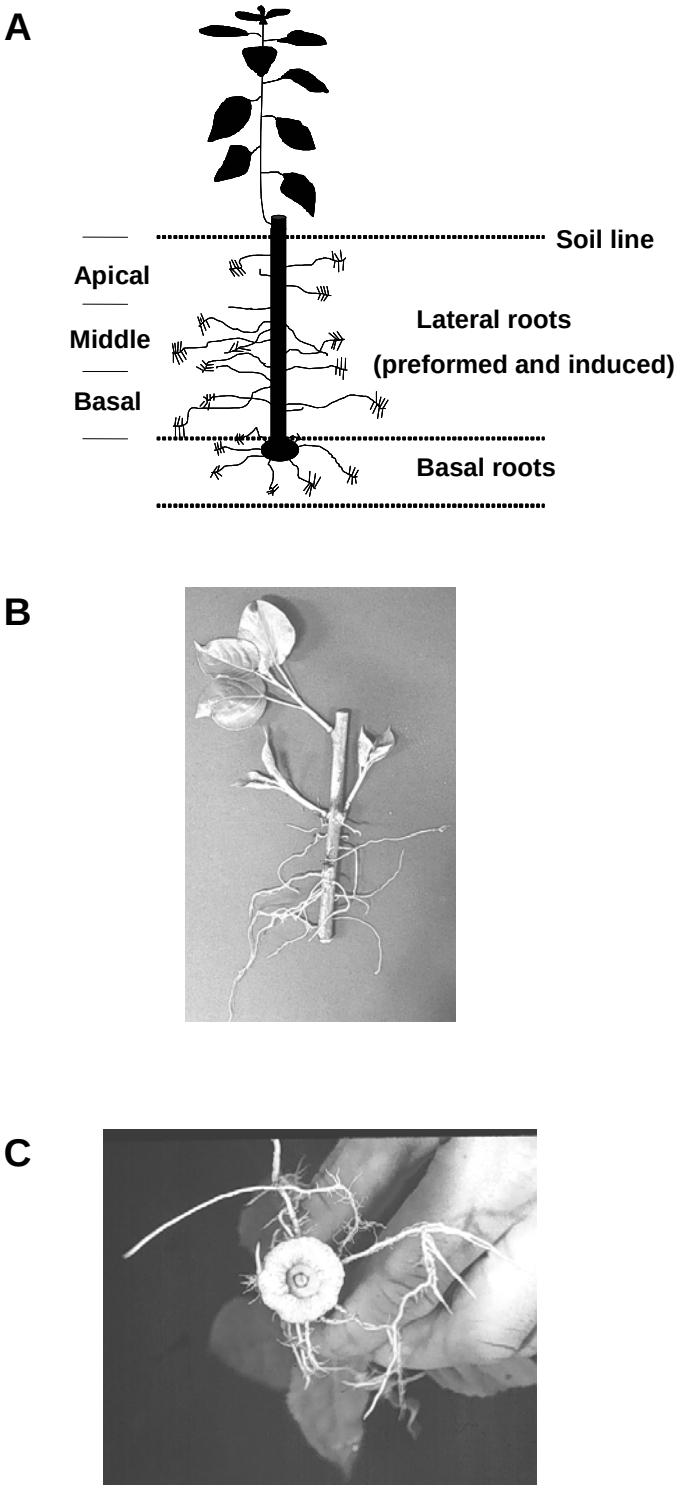

Figure 1. Lateral and basal adventitious root (AR) types of *Populus*, along with apical, middle, and basal sections of the hardwood cutting (A), photograph of lateral rooting along the length of the cutting (B), and photograph of basal root development from callus cells at the base of the cutting (C). Photographs by Edmund O. Bauer.

have made *Populus* a suitable potential alternative for the production of ethanol (cellulosics for biofuels) and electricity (biomass for bioenergy). In addition to high water usage rates and fast growth [36], the extensive and variable root systems of *Populus* are important for contributing environmental services that are necessary for long-term ecosystem sustainability across multiple temporal and spatial scales.

Adventitious root formation is crucial for plantation development, regardless of application. Extensive rooting decreases costs associated with establishment and vegetation management by reducing time period to crown closure and increasing operational flexibility of planting schedules across varying environmental conditions [17]. In addition, adventitious rooting affects stocking levels and yields at harvest given its influence on tree survival. There is an opportunity to select *Populus* genotypes with some combination of both AR types for specific applications. Both root types may enhance phytoremediation and associated technologies. Shallow roots are ideal for interception of contaminants in irrigation sources, while sinker root types are vital for applications where hydraulic control is necessary. Windthrow problems affecting fiber production in the Pacific northwestern United States can be reduced by selection and deployment of specific *Populus* clones growing deep roots from either lateral or basal root systems for increased anchorage. Trees established at close spacings as energy feedstocks may be more productive with a combination of lateral and basal roots necessary for tolerance to inter-tree root competition.

Reviews of the anatomy, physiology, and genetics of AR formation in *Populus*, along with external factors affecting such rooting, are described in subsequent sections of this chapter. The practical importance of selecting root types for applications such as environmental benefits, fiber, and energy is described in the final section, with special attention to how rooting enhances economic and environmental sustainability.

Anatomy of rooting

Lateral rooting

There are two categories of lateral roots in *Populus* cuttings: (i) preformed roots formed from primordia developed during the previous growing season and (ii) induced roots formed as a response to external stimuli during growth and development. Both types of lateral ARs are thought to be under strong genetic control [19]. Root initiation from latent preformed primordia depends on age of the primordia, presence of vegetative buds, and stratification [37]. The preformed primordia form early and are active throughout the growing season [22,37,38], with older primordia

located toward the base of the shoot [24,39,40]. Induced root primordia orginate near vascular tissues within the cambium, phloem, and pericycle [21]. Dormancy of the root primordia follows cessation of vascular cambium activity during winter months [24,38].

Basal rooting

There is less knowledge about the anatomy of basal ARs than lateral ARs in *Populus*. Processing dormant hardwood propagules from parent shoots involves cutting the stems into desirable lengths, which creates wound sites at the apical and basal ends of the propagule. Basal rooting of *Populus* begins with the development of a parenchymous mass, i.e., callus, at the base of the cutting in response to this wounding (Fig. 1). Callus cells first differentiate into root primordia and second into basal ARs in response to external stimuli [21].

Physiology of rooting

Physiological status of cuttings

Deployment success is dependent on the physiological status of cuttings, because shoot and root growth are subject to levels of endogenous resources available [41,42]. Cuttings utilize carbohydrates, both starch and sugar, and proteins stored in the stem to fuel the initial burst of shoot growth from preformed buds. While initial shoot growth is primarily dependent on stored C in the stem, the young roots gain energy from newly expanding and photosynthesizing leaves. Overall, *Populus* cuttings are entirely dependent on the development of the root-shoot vascular connection. Establishment will fail if cuttings are unable to produce ARs from preformed or induced primordia, expand their leaves from dormant buds, and connect vascular tissues between roots and shoot [43]. This interdependence between root and shoot developmental systems continues throughout the life of the tree as the root is completely dependent on C assimilates produced in the shoot and the shoot is largely dependent on hormones synthesized in the root for plant regulation [44].

Carbon allocation

Populus photosynthetic rates are among the greatest for woody plants and are associated with high net primary productivity and fast growth [43,45]. Carbon allocation is important for adventitious rooting of *Populus* because growth and development of the entire tree depends on production of C in the photosynthetic organs and subsequent distribution within the tree via phloem to the root and shoot systems [46]. Carbon allocation includes: (i) partitioning among plant organs, i.e., roots, stems, and leaves, (ii)

consumption via respiration and active nutrient uptake, (iii) production of defense chemicals, (iv) response to injury and repair of tissues, (v) losses to the rhizosphere, and (vi) storage [47]. The process involves movement of C from sources to sinks. The source-sink relationship is highly variable based on plant age, environmental conditions and stresses, including nutrient availability, water, light, temperature, diseases, and insects, seasonal changes such as budburst and bud dormancy, and genetic variability (Fig. 2).

Leaf developmental stage affects C allocation within *Populus* trees. For example, young leaves assimilate C but require additional imports for growth; however, as leaves age they become exporters with C products sent to leaves, stems, and roots [48]. Older leaves and leaves of sylleptic branches mostly provide C resources to the stem and root [43,48]. Carbon transport from mature leaves downward is important for adventitious rooting of *Populus*, because the whole-tree C balance is easily disrupted during crown damage, insect attack, or disease defoliation events. Heavily defoliated *Populus* trees must allocate C to leaf replacement, which limits the resources provided for root growth and storage [43]. Therefore, the ability of specific clones to produce sylleptic branches that provide C to the lower portion of the tree may be beneficial and, as such, are often considered in breeding and selection programs [48-50].

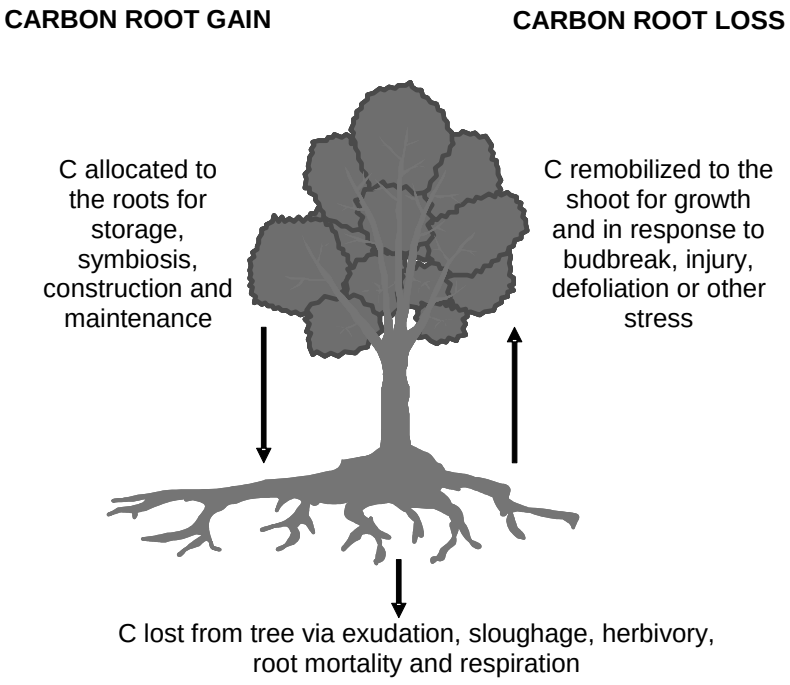

Figure 2. *Populus* above- and below-ground carbon (C) processes showing general source-sink allocation patterns.

Seasonal patterns of C storage as carbohydrates and lipids occur within the tree and affect AR formation of *Populus*. Terminal budset is the most critical phenological mechanism regulating carbohydrate allocation to the roots, with greater amounts of nonstructural carbohydrate storage into the roots during budset [51]. In addition, tree age affects the amount of C stored in the roots, with younger plants storing more C than older trees. As plants age the increased ability of the stem to store more carbohydrates decreases overall allocation to the roots [47,52]. Stored reserves in the roots are used to fuel spring growth, as well as to aid recovery from catastrophic environmental injury [43,53].

Root structure, growth, and mortality

Populus root systems display a strong genetic effect, i.e., are genotype-specific, and are quite variable across environments. *Populus* root systems from cuttings form an extensive network of coarse horizontal, vertical sinker, and fine roots that are developed from lateral and basal ARs [43,54]. For example, Friend et al. [55] reported lateral roots extending up to 2.8 m from the cuttings of 1-year-old trees of *P. trichocarpa* × *P. deltoides* hybrids. In the same experiment, they reported 2-year-old trees with roots extending greater than 4 m horizontally and 1.5 m vertically [55]. Furthermore, allometric ratios between root and shoot systems are important for biomass production, regardless of application [56]. Reduced root:shoot ratios may be desired under ideal growing conditions. However, an increased root C investment is beneficial to survival under stressful environments, because larger root systems can display greater spatial distribution and water/nutrient foraging capacity [18,54]. Under growing conditions with adequate fertilizer and moisture, biomass is thought to be shifted to favor aboveground tissue over belowground tissue, resulting in decreased root:shoot ratios [43,53]. Yet such shifts were negligible for two *P. deltoides* clones in the Southeast, as developmental stage of the plantation accounted for the changes in root:shoot ratios rather than potential indirect effects of fast growth [56]. Regardless, *Populus* trees invest a great deal of C and nutrients into actively cycled fine root production (< 1.0 mm) as these roots primarily provide uptake of water and nutrients required for growth [43,57,58].

Fine root demography has been shown to be quite plastic to environmental conditions, including patches of nitrogen (N) and water. Pregitzer et al. [59] determined absorbing fine roots exhibited exceptionally fast growth rates (> 10 mm^{-1} d^{-1}) and were highly responsive to regions of moisture and fertility for *P. deltoides* × *P. nigra* hybrid cv. 'Eugenei'. Relative to conditions without patches, root growth and life span were

increased in a mixed hardwood forest containing *P. grandidentata* [60] and root mass was increased for *P. trichocarpa* females, *P. deltoides* males, and their F_1 and F_2 hybrids [61]. Soil depth, root diameter and age, N fertilization, and season affected fine root survival of *P. deltoides*, while increased N and median root life span were positively correlated [62]. Although *Populus* trees respond to N amendments with increased growth [56, 63], recommendations for N fertilization, silvicultural systems, length of growing season, and clonal material vary by region of deployment and specific application [64-66].

Genetics of rooting

Quantitative genetics

The broad variation in AR formation of *Populus* is under strong genetic control and is thus responsive to selection [16,67-69]. However, more information about the quantitative genetics of rooting is needed to increase the success of *Populus* for a variety of applications [70,71]. Heritability estimates from replicated clonal tests at multiple locations, as well as genetic, phenotypic, and environmental correlations between root and shoot developmental systems, have been used to predict selection gains associated with increased rooting ability. Broad-sense heritability estimates for rooting traits such as dry mass, number, and length have ranged from 0.15 to greater than 0.80 [15-17,67-69]. Overall, substantial variation in adventitious rooting of *Populus* has been attributed to inherent additive genetic effects, positional effects and environmental preconditioning of the parent shoots (i.e., C-effects), and genotype × environment interactions [16,17,67,68].

Adventitious rooting of specific *Populus* genotypes is often difficult to predict given heterogeneous growing conditions across varying contaminant levels in phytotechnology applications [72,73] and across sites in fiber and energy applications [71,74]. Such genotype × environment interactions have led to the need for selection of generalist genotypes performing well across a broad range of unpredictable environmental variation or specialist genotypes well-adapted for specific conditions [6,17]. For example, rooting of *Populus* clones across three sites in Iowa and Minnesota, USA, was relatively stable in the face of contrasting weather and soil conditions (Fig. 3) [17]. In contrast, there was broad variation in rooting of eight *Populus* clones irrigated with either municipal solid waste landfill leachate or nutrient amended well-water for two growing seasons in northern Wisconsin, USA [50]. An *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* F_1 hybrid (clone NC14104) was identified that exhibited greater root dry mass when irrigated with leachate, compared with two clones that rooted better with well-water and five clones that were generalists (Fig. 4). There is a clear need for

continual testing and selecting of both generalist and specialist genotypes, regardless of desired application. Further information about common genotype × environment interactions influencing AR formation of *Populus* is described in the section EXTERNAL FACTORS AFFECTING ROOTING, below.

Molecular genetics

Quantitative genetics provides necessary information about the genetic basis of AR formation in *Populus*, but the complexities of this tissue system and the laborious task of excavating roots in traditional clonal tests makes acquisition of such data difficult [17]. Thus, there is a need for molecular genetics combined with quantitative genetics ("molecular breeding") to provide new information beyond the genomic group and clone level [75-77].

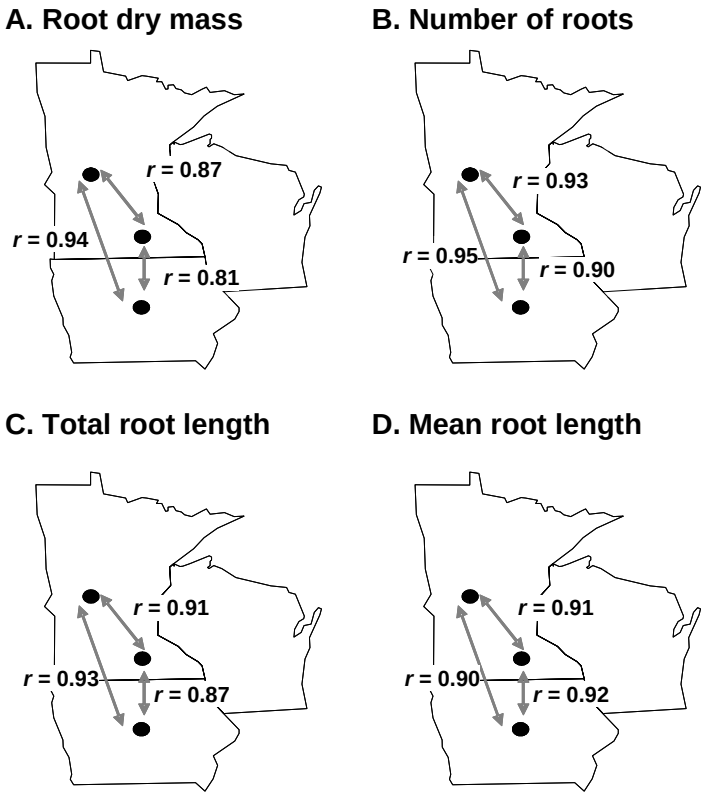

Figure 3. Phenotypic correlations among Ames, Iowa, USA (42.0 ˚N, 93.6 ˚W); Waseca, Minnesota, USA (44.1 ˚N, 93.5 ˚W); and Westport, Minnesota, USA (45.7 ˚N, 95.2 ˚W) for root dry mass (A), number of roots (B), total root length (C), and mean root length (D) in an experiment testing 21 *Populus* clones in their ability to develop roots from dormant hardwood cuttings (n = 21). All correlations were significant at $P < 0.0001$. Reprinted with permission from Can. J. For. Res. [17].

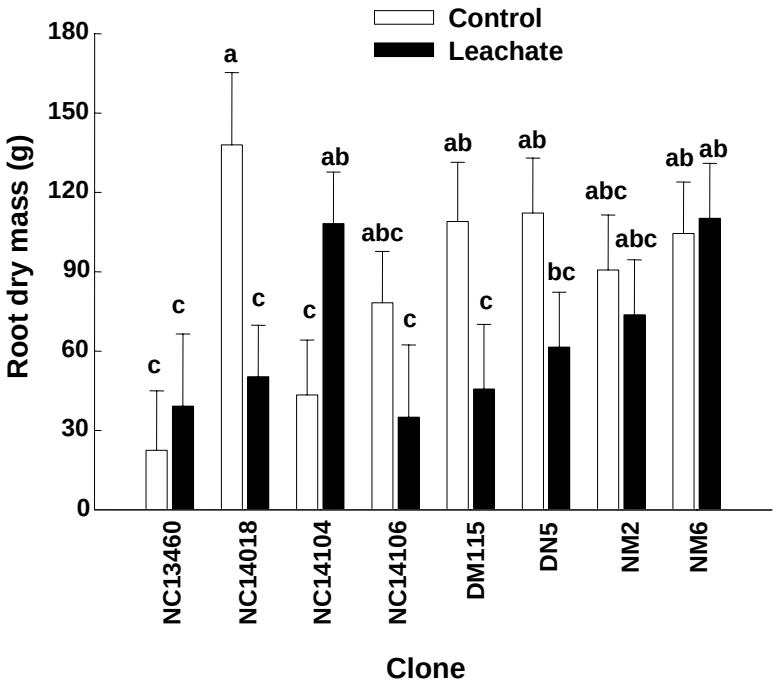

Figure 4. Root dry mass of eight *Populus* clones 14 months after planting following once-weekly landfill leachate irrigation during the 2005 (3.8 L tree^{-1} week^{-1}) and 2006 (22.7 L tree^{-1} week^{-1}) growing seasons. The control treatment was nutrient amended well-water applied at a volume equal to that of the leachate. Bars represent the mean (n = 3 to 8 trees) and error bars are one standard error. Bars denoted by different letters were different at $P < 0.05$. Reprinted with permission from For. Ecol. Manage. [50].

Molecular genetic analyses of *Populus* provide information about the: (i) chromosomal location of each quantitative trait locus (QTL) affecting the trait, (ii) magnitude of effect of each QTL on the observed phenotype, (iii) mode of gene action at each QTL (additive, dominant/recessive, overdominant), (iv) effect of interactions among different QTL's (epistasis), and (v) parental source of beneficial QTL alleles [75; page 185]. For example, Bradshaw et al. [78] developed a linkage map in an F_2-pedigree population with inter-American (*P. deltoides* × *P. trichocarpa*) parentage. Adventitious rooting was one of the traits used to segregate this F_2 population; therefore, the map along with markers could be used to directly clone genes responsible for AR initiation in *Populus*. More recently, molecular genetics techniques were successfully used to create linkage maps of many *Populus* species [79,80] and sequence the entire *P. trichocarpa* genome [81]. Such sequencing has profound implications for the manipulation of adventitious rooting for environmental benefits, fiber, and energy. Future research devoted to AR formation should take advantage of "molecular breeding" along with traditional methods of analysis [75]. A more

detailed description of the molecular genetics of *Populus* adventitious rooting is described in Chapter IV of Busov et al.

Tree improvement

The success of *Populus* hybridization has ranged from complete compatibility to complete incompatibility resulting from prefertilization and postzygotic crossing barriers and hybrid inviability [82-84]. Strategic breeding decisions have led to substantial inter- and intra-sectional crosses between and within sections *Aigeiros* and *Tacamahaca* [9,85]. Genomic groups from these sections have exhibited variable adventitious rooting responses. For example, Zalesny and Wiese [86] tested 22 *Populus* clones belonging to six genomic groups for number and dry mass of roots, and they reported differences between groups with at least 50% *Tacamahaca* parentage versus those with pure *Aigeiros* parentage. Specifically, parental shoots were collected at different times throughout the dormant season and processed into cuttings from apical, middle, and basal sections of the shoots. Number and dry mass of roots were greatest for apical cuttings of *Tacamahaca* genotypes, while their *Aigeiros* counterparts rooted better from middle and basal cuttings [86].

The aforementioned genetic variation among and within *P. deltoides*, *P. trichocarpa*, *P. nigra*, and *P. suaveolens* has led to consistent strategic species selection in the midwestern and Pacific northwestern United States [10,87]. An overarching goal in Midwest *Populus* breeding is to incorporate elevated growth and insect/disease resistance of native *P. deltoides* genotypes with increased adventitious rooting ability from the other three species [17]. For example, *P. deltoides* roots erratically from dormant hardwood cuttings in the Midwest [17,19,88], while *P. trichocarpa* and *P. suaveolens* root very well but are highly susceptible in this region to some pathogens and pests (Table 1).

Operationally, there is a need for specific crosses and designs to improve rooting while maintaining adequate growth and levels of pest and disease resistance. Two common breeding strategies used to achieve this goal are F_1 hybrid production and first generation backcross (BC_1) hybrid production [11].

External factors affecting rooting

Propagation conditions

Processing

Adventitious rooting is associated with larger *Populus* cuttings because of increased stored carbohydrates [41,42,51], number of preformed root

primordia [37], and overall vigor of the young trees from which the cuttings are made [89-91]. Recent studies have shown root dry mass is nearly always positively correlated with increased size of cuttings; however, number of roots fails to consistently show a similar correlation with cutting size (Table 2). Thus, size of cuttings has some effect on root initiation and a substantial influence on root growth. Meristematic activity of primordia decreased with age [22,37,39]; therefore, increased adventitious rooting of *Populus* is attained using cuttings from one-year-old shoots versus older stems [92]. For example, Houle and Babeux [93] tested the percentage of cuttings rooted of *P. balsamifera* at different ages and reported ranges of 60 to 100% for young trees and 7 to 100% for old trees.

In addition, the original position on the parent shoot from which cuttings are made affects AR formation of *Populus*, with a general trend of increasing root growth from cuttings originating closer to the base of the parent shoot [69,94]. For example, number of roots and root length were greater for *P. trichocarpa* cuttings made from the bottom quarter of the original shoot relative to rooting on sections from the top and middle two quarters [95]. Similarly, basal cuttings of *P. ×canadensis* hybrids and *P. trichocarpa* had greater root length than apical cuttings for all genotypes [96]. Likewise, cuttings made from the basal third of parent shoots across 21 *Populus* clones field-tested at three sites in Iowa and Minnesota, USA, exhibited greater root dry mass, number of roots, and total root length than those from apical and middle positions (Table 3) [19].

Table 2. Effect of cutting size on dry mass and number of roots during studies testing the effects of external factors on adventitious rooting of *Populus*.

Study	Cutting mass	Root dry mass[z]	Number of roots
Zalesny et al. [3]			
Experiment 1	1.1 – 7.7 g	***	ns
Experiment 2	1.4 – 9.0 g	ns	ns
Experiment 3	1.1 – 7.5 g	***	ns
Zalesny and Wiese [86]			
Full model	0.5 – 13.9 g	***	***
Reduced model	0.5 – 13.9 g	***	ns
Wiese et al. [110]	1.2 – 7.6 g	***	ns
Zalesny et al. [124]	2.5 – 14.8 g	***	***
Zalesny et al. [19]	0.3 – 1.6 cm[y]	***	***

[z] *** = cutting mass significantly affected rooting; ns = cutting mass did not significantly affect rooting.

[y]Cutting diameter was used instead of mass in the analyses of covariance.

Table 3. Root dry mass, number of roots, and total root length (adjusted for cutting diameter) in an experiment testing for differences in rooting ability among stem cuttings of five genomic groups[z] of *Populus* based on their positions on the shoot system of the parental stool plant. Mean ± standard error (n = 756 cuttings for each combination of year and position). Means within a column followed by the same letter are not significantly different (Fisher's protected LSD, α = 0.05). Reprinted with permission from Silvae Genet. [19].

Position[y]	Root dry mass (mg)		Number of roots		Total root length (cm)	
	2001	2002	2001	2002	2001	2002
Apical	3.6 ± 0.3b	3.4 ± 0.3c	2.1 ± 0.1b	2.7 ± 0.2c	4.4 ± 0.4b	4.3 ± 0.4c
Middle	3.4 ± 0.3b	4.5 ± 0.3b	2.2 ± 0.1b	3.3 ± 0.2b	4.3 ± 0.4b	6.1 ± 0.4b
Basal	5.4 ± 0.3a	5.4 ± 0.3a	3.2 ± 0.1a	3.8 ± 0.2a	7.0 ± 0.4a	7.5 ± 0.4a

[z]Genomic groups are: BC = (*P. trichocarpa* × *P. deltoides*) × *P. deltoides*, D = *P. deltoides*, DM = *P. deltoides* × *P. suaveolens* subsp. *maximowiczii*, DN = *P. deltoides* × *P. nigra*, NM = *P. nigra* × *P. suaveolens* subsp. *maximowiczii*.

[y]Cuttings made from the upper, middle, and lower third of the parental stool plant are designated apical, middle, and basal, respectively.

The combination of specific genotypes and positions also contributes to the broad variation in rooting ability of *Populus*. For example, the best rooting of clones with (*P. trichocarpa* × *P. deltoides*) × *P. deltoides*, *P. deltoides*, and *P. deltoides* × *P. nigra* was with cuttings from basal shoot positions, while this basal superiority diminished for *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* and *P. nigra* × *P. suaveolens* subsp. *maximowiczii* hybrids [19]. Along with *P. trichocarpa* genotypes, root dry mass on cuttings from apical and middle positions was better than basal positions for *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* and *P. nigra* × *P. suaveolens* subsp. *maximowiczii* clones collected seven times from December to April in northern Wisconsin, USA (Fig. 5) [86]. Overall, clones with exclusive parentage from the section *Tacamahaca*, i.e., *P. trichocarpa*, and *P. suaveolens* subsp. *maximowiczii*, exhibited the apical superiority, while those from the section *Aigeiros*, i.e., *P. deltoides* and *P. nigra*, had better rooting with basal cuttings only.

The time during the dormant period when parent shoots are harvested from the field and made into cuttings is another critical processing component that supports or hinders AR formation of *Populus* [86,93]. Parent shoots need a sufficient chilling period for physiological dormancy, i.e., stratification, to properly break bud dormancy and achieve adequate rooting and growth [15,97,98]. For example, when cuttings of *P. balsamifera* were collected from October through April, the percent rooting and number of roots per cutting was greater when cuttings were collected after trees became dormant than those collected before chilling requirements had been met [99].

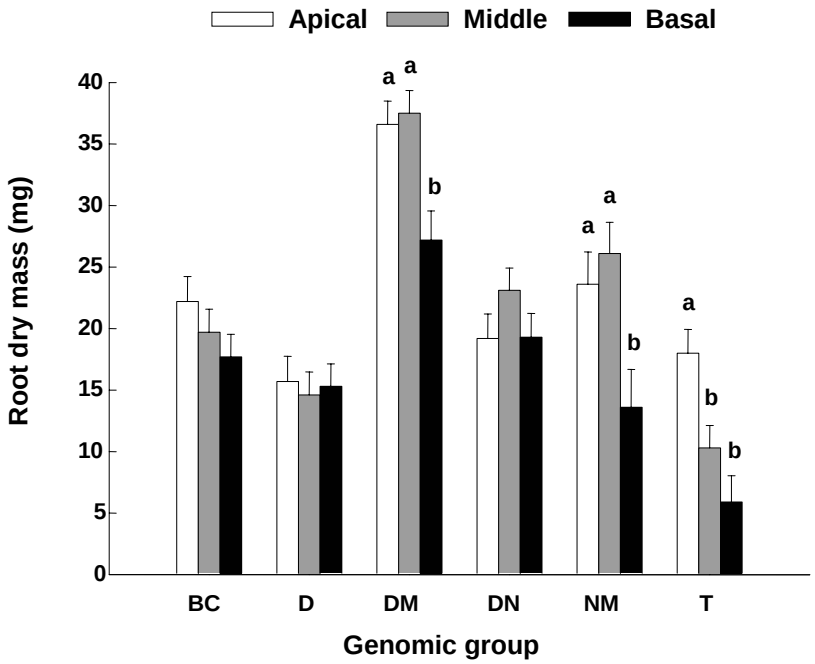

Figure 5. Root dry mass of *Populus* cuttings belonging to six genomic groups taken from apical, middle, and basal positions of shoots of the parental stool plant. Genomic groups: BC = (*P. trichocarpa* × *P. deltoides*) × *P. deltoides*, D = *P. deltoides*, DM = *P. deltoides* × *P. suaveolens* subsp. *maximowiczii*, DN = *P. deltoides* × *P. nigra*, NM = *P. nigra* × *P. suaveolens* subsp. *maximowiczii*, T = *P. trichocarpa*. Bars represent the mean adjusted for cutting dry mass and error bars are one standard error. Bars within a genomic group denoted by different letters are significantly different (Fisher's protected LSD; $\alpha = 0.05$; LSD: BC, D, DM, DN, and T = 5.1, NM = 7.3). Reprinted with permission from Silvae Genet. [86].

Likewise, the percent rooting of *P. deltoides* collected in March (41 °N, 93 °W) was three times greater than those from December and twice that of November [92]. Nevertheless, collection of shoots too late in the dormant season reduces potential to form ARs due to translocation of reserves to buds for aboveground growth [100,101]. Farmer [102] (33 °N, 90 °W) reported *P. deltoides* collected in late-January expressed higher rooting percentages than those collected in December, February, or March. Zalesny and Wiese [86] collected parent shoots of 22 *Populus* clones from December through April (45 °N, 89 °W) and reported a general trend of the best root dry mass and number of roots from late February and early March collection dates. Overall, adventitious rooting based on time of harvest was genotype-specific [86].

Storage

Storage duration and temperature affect AR formation of *Populus* cuttings. While cuttings of some woody perennial species can be stored just

above 0 °C for nearly twelve months without significant reduction in viability [103], *Populus* cuttings should be stored for less than six months at temperatures between 3 °C and 7 °C. Extended cutting storage duration at temperatures above 7 °C may result in root initiation during storage, increased respiration, microbial attack, and eventual dessication. Longer time periods require storage at subfreezing temperatures. For example, Phipps et al. [104] tested for differences in root and shoot growth in three *Populus* clones after one year storage at -7 °C and concluded cuttings should be subjected to a warming period of at least two weeks at 3 °C, and then soaked in water until root emergence.

Pre-planting treatments

Soaking *Populus* cuttings in water under dark conditions and ambient temperatures promotes adventitious rooting [105]. Rooting is optimized when cuttings are soaked until root emergence but not beyond, which includes lateral root nodule formation and/or the beginning of callus development at the base of the cutting [104,106]. Soaking greatly enhances the survival and growth of *Populus* during establishment [96,107,108]. For example, DesRochers and Thomas [105] reported significantly greater rooting percentages for cuttings soaked 8 or 14 d versus not being soaked for *P. deltoides* × *P. nigra* cv. 'Walker', *P. balsamifera* × *P. simonii* cv. 'P38P38', *P. balsamifera* × *P. deltoides* cv. 'Jackii10', and *P. laurifolia* × *P. nigra* cv. 'Berlin42' hybrids.

The removal of floral and vegetative buds differentially affects AR formation of *Populus* from young scions of older trees capable of flowering and one-year-old shoots [109,110]. Farmer [102] removed all floral buds from *P. deltoides* cuttings and observed twice as many roots per cutting as on cuttings with intact floral buds. Wiese et al. [110] tested the effects of 0%, 50%, and 100% vegetative bud removal on adventitious rooting of 10 *Populus* hybrids and reported removal of less than or equal to 50% of the buds did not affect overall establishment. Bud removal had no effect on root biomass but did influence root initiation, which are processes regulated by different mechanisms [47,70,111]. While root biomass is a result of increased reserve allocation over time, short-term meristematic activity governs root initiation [105,110]. Overall, *Populus* cuttings with more vegetative buds initiate greater numbers of roots than those with fewer buds [37,98,112].

Site conditions

Soil moisture

A combination of precipitation and irrigation contributes to the soil moisture content of *Populus* plantations in regions such as the midwestern

United States, while drier areas such as some plantations in the Pacific Northwest [87] and Southeast [113] require extensive irrigation. Excessive water causes conditions of poor aeration and reduced growth, while lack of water leads to desiccation and death [44]. Allen and McComb [92] tested *P. deltoides* rooting in five soil moisture treatments ranging from below field capacity to saturated and reported the percentage of cuttings rooted (0 to 80%) and number of roots per cutting (2 to 20) increased with increasing soil moisture up to the point of saturation. The best adventitious rooting of 21 *Populus* clones belonging to five genomic groups established at three sites in Iowa and Minnesota, USA, during two field seasons occurred on cuttings receiving sufficiently-dispersed precipitation rather than equal volumes supplied less frequently [88]. Rooting was most successful when there were no more than three days without some precipitation event.

Extensive development of AR systems contributes to efficient drought resistance mechanisms in some *Populus* species [114,115]. For example, the larger and more efficient root system of a *P. deltoides* × *P. nigra* 'DN' F_1 hybrid was able to occupy more soil space and thus capture higher levels of available water relative to a *P. trichocarpa* × *P. deltoides* 'TD' F_1 hybrid [116]. The DN clone allocated more C to the roots during periods of drought, in addition to sustaining efficient stomatal regulation. Likewise, the maximum root length of three *Populus* clones (*P. balsamifera* cv. 'B3'; *P. trichocarpa* cv. 'T6'; *P. nigra* × *P. nigra* cv. 'NE308') was nearly seven times greater in soils containing adequate water versus those in drier conditions [117]. Regardless of drought resistance mechanisms, there is a need for extensive AR formation to supply moisture demands of the trees, especially given high permeability in sandy soils and periodic drought conditions causing clay soils to become impenetrable [118].

Soil and air temperature

Proper soil and air temperatures facilitate *Populus* AR formation [22], while temperature-driven root metabolism governs root growth and uptake [44]. Combining cold soil temperatures and warm air temperatures may reduce the vigor of developing trees given their inability to conduct water fast enough to meet leaf and shoot transpirational demands [119-121]. Cold soil temperatures also reduce cell division and activity within root primordia [22], while accelerated respiration levels at high temperatures [122] deplete stored reserves in cuttings and often result in death of young trees [123].

While AR formation of *Populus* is generally sustained at temperatures above 10 °C [107,119,121], a more meaningful approach has been the evaluation of rooting based on a thermal index defined by the accumulation

of belowground growing degree days (GDD) [88,124]. Growing degree days are the sum of the mean temperature in a 24-h period minus a base temperature that is the threshold supporting growth (i.e., 10 °C) [124,125]. Zalesny et al. [88] reported above-average root dry mass, number of roots, and root length for 21 *Populus* genotypes grown in the North Central United States where the minimum soil temperature on four consecutive days was above 14 °C, and precipitation was sufficiently-dispersed. The base soil temperature threshold for *Populus* rooting in this region is 14 °C. In addition, there is broad variation in adventitious rooting based on the response of specific clones and genomic groups to the accumulation of soil temperatures. For example, Zalesny et al. [124] tested the response of two *P. deltoides* and four *P. deltoides* × *P. suaveolens* subsp. *maximowiczii* clones to varying soil temperatures in northern Minnesota, USA, and reported the greatest root dry mass for *P. deltoides* at belowground GDD ≥ 163 and for the hybrids at belowground GDD ≤ 173, assuming the previous base temperature of 10 °C (Fig. 6).

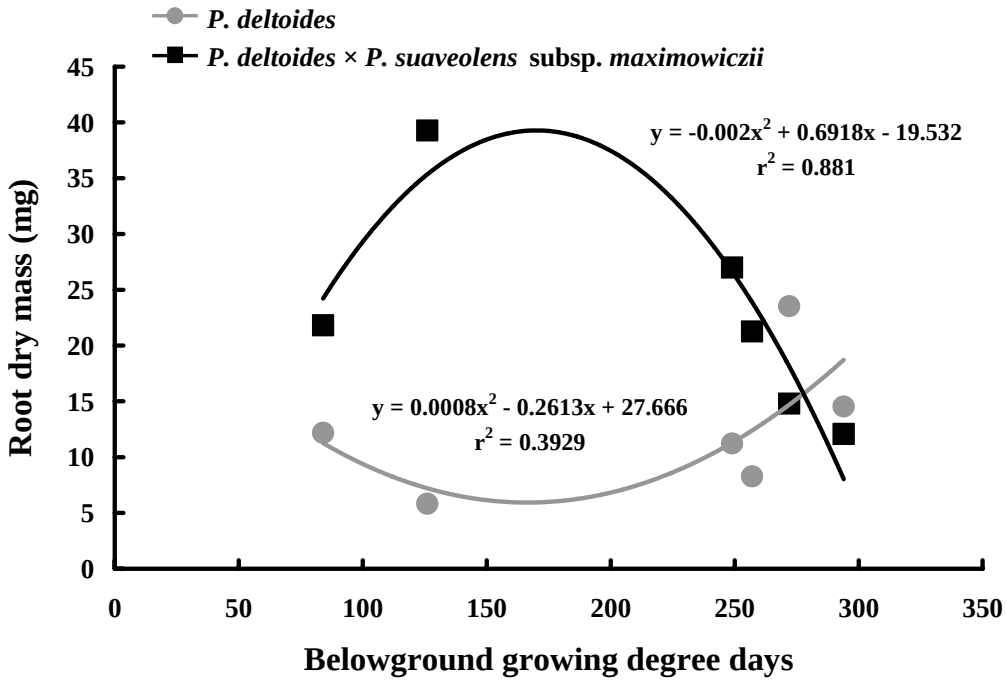

Figure 6. Least-squares regression analysis of root dry mass on belowground growing degree days (GDDs) of two *Populus* genomic groups [*P. deltoides* 'D' and (*P. deltoides* × *P. suaveolens* subsp. *maximowiczii*) 'DM'] when planted as dormant hardwood cuttings. Data points represents the mean of 20 cuttings for the D clones and the mean of 40 cuttings for the DM clones. Reprinted with permission from Silvae Genet. [124].

Practical uses of different root types important for environmental sustainability

Environmental benefits

Adventitious rooting of *Populus* is well suited for specific applications such as nutrient and chemical removal in phytoremediation and riparian buffer systems. Strong and deep root systems of *Populus* securely anchor windbreaks and shelterbelts, while extensive and shallow roots control erosion. All of these ecosystem services provide sequestration of carbon dioxide released into the atmosphere from fossil fuel consumption and land use change, which are significantly affecting our climate.

Phytoremediation involves the utilization of metabolic pathways of green plants for the removal, transformation, or stabilization of contaminants in soil and groundwater to manage environmental waste [126]. These plant-based systems succeed when plant growth promoting factors, i.e., nutrients, light, water, aerobic conditions, exceed the growth inhibiting factors, i.e., heavy metals, volatile organics, salts, anaerobic conditions, contained in the medium or irrigation source. Of the woody species currently investigated for remedial applications, *Populus* has been utilized to the greatest extent. The dimorphic root systems produced adventitiously on cuttings provide lateral roots with extensive fine root surface area crucial for water and nutrient uptake and deep sinker roots for anchorage and penetration to zones of saturation, even groundwater [127]. Dynamic fine root processes increase remediation success and increase microbial populations with high turnover rates contributing to soil C via exudation, mortality, and root sloughage [54]. Fine roots are the primary regions of mycorrhizal associations that increase the potential foraging area for nutrient and chemical uptake, as well as sites of chemical transformations.

Typically, greater water use efficiency is a desired trait in agronomic or horticultural settings, but the ability of *Populus* trees to tap into deeper and more permanent sources of water increases the success of groundwater remediation systems. The deep *Populus* root systems extending several meters into the ground increases hydraulic control and the movement of the soil solution into the xylem [36, 128]. Genotype × environment interactions in remediation systems govern plant responses ranging from phytotoxic effects including mortality to elevated viability and tolerance to contaminants [72].

Riparian buffer strips are a major agroforestry practice employing plant-based remediation for non-point source pollution at the interface of agricultural fields and water resources [129]. Extensive *Populus* root systems within the multi-zoned, species-diverse, riparian buffers slow the movement

of materials, trap sediments, and reduce nutrients and chemicals discharged into waterways by means of plant uptake, microbial activity, and other processes [30]. Additional on-farm conservation practices enhance ecosystem protection and pollution reduction to surface and subsurface water.

Populus stands utilized for windbreaks and shelterbelts provide a barrier protecting against wind damage to livestock, property, and fields. Typically one or more rows of *Populus* trees are planted as windbreaks to shield livestock, homes, buildings, and property from the destruction and battery of cold winter winds and summer heat [130]. Shelterbelts provide a barrier to the weather sweeping across agricultural fields, thus increasing soil stabilization and reducing erosion [130]. In these applications, it is essential the trees have a combination of deep sinker root systems for anchorage to prevent windthrow and wide-spreading lateral roots exhibiting extensive networks of shallow roots for soil stabilization and erosion control.

Fiber and energy

Decades of research on *Populus* has elucidated the silvicultural, genetic, physiological, and plant health knowledge making these trees preferred short rotation woody crops for fiber and energy. In these systems, trees are planted at spacings typically ranging from 2 to 4 meters among and within rows to achieve greater harvestable biomass, with regional variations in the silvicultural systems employed [113]. Inter-tree competition for water and nutrient resources occurs as a result of such high density planting and extensive root system development [131,132]. The existence of this competition provides an opportunity to select *Populus* genotypes with enhanced AR formation to improve fiber and energy feedstock production and to increase tolerance of environmental stresses such as resource competition and windthrow. We believe trees established at close spacing may be more productive with a combination of lateral and basal ARs necessary to withstand inter-tree root competition while increasing the horizontal and vertical distributions of the overall root systems. For example, windthrow can cause devastating damage to plantations in regions such as the Pacific Northwest as roots are concentrated near irrigation emitters and overall root system distribution and mass are decreased. Such production systems with adequate irrigation and fertilization reduce the need for high C allocation to extensive root system development. Regardless, the ideal root system should provide lateral and fine roots required for resource uptake and deep sinker roots for strong anchorage against severe wind threats. Greater water use efficiency in selected clones is desired in heavily irrigated systems to reduce production costs.

Conclusions

Populus species have been used for numerous applications with environmental and economic benefits. The two AR types from hardwood *Populus* cuttings are: (i) lateral roots developing from either preformed or induced primordia along the length of the cutting and (ii) basal roots differentiating from callus at the base of the cutting in response to wounding imposed by processing the parent shoot into propagules. The ability of *Populus* cuttings to form ARs from preformed and induced primordia, as well as from callus cells is a key trait in the success of these short rotation crops when utilized for environmental benefits, fiber, and energy. Given the importance of AR formation to plantation establishment and subsequent development, adventitious rooting has been and will continue to be an important early selection criterion in *Populus* breeding, regardless of end use. Breeding and selecting *Populus* genotypes with favorable adventitious rooting ability involves a range of complexities from testing in the greenhouse to the field, as well as simply using survival as a surrogate for rooting versus conducting excavations of entire root systems. While current research involves studies within this broad scope, future research should focus on the development of new genotypes and subsequent selection of unique clones exhibiting a combination of lateral and basal AR types capable of optimal rooting in varying environments for specific applications.

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