AUXIN-PRODUCING ENDOPHYTE INOCULATION IMPROVES EARLY HEIGHT GROWTH OF SELECTED HYBRID POPLAR HARDWOOD CUTTINGS IN A MICHIGAN FIELD TRIAL.

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ABSTRACT

A group of diazotrophic microorganisms (endophytes) has been shown to produce auxins in culture and to stimulate root and shoot development in *P. trichocarpa* and *P. deltoides* hybrid cuttings in greenhouse trials. Here we inoculated dormant, hardwood cuttings of three diverse poplar taxa with an auxin-producing endophyte, planted them in a field trial in Upper Michigan, and compared their vigor and height growth with uninoculated cuttings for 55 days. A *Populus alba* X *P. grandidentata* hybrid did not root and all but one of the 120 cuttings died. Similar sets of *P. deltoides* (DD) hybrids and a P. *deltoides* X *P. nigra* hybrid (DN) survived and grew well. The DN hybrid grew 48% taller than the DD hybrids, demonstrating inter-species hybrid vigor. Inoculated cuttings of both DD and DN hybrids grew 14% taller than uninoculated cuttings of each taxa during the 55 days of this trial. If endophyte inoculation translates into long-term yield improvement, this would significantly improve the finances of poplar short rotation production systems. Increase yields without the addition of chemical fertilizers would also benefit the energy balance of poplar crop production.

INTRODUCTION

Hybrids from the genus *Populus* (poplars) make excellent crops for short rotation forestry systems because they are broadly adapted, easily propagated, and fast growing. Commercial poplars are usually propagated clonally and planted as unrooted dormant hardwood stem cuttings (*cuttings*). This maintains the genetic characteristics obtained through advanced breeding programs and is substantially less expensive than establishing plantations using seedlings or stecklings[1]. The genus *Populus* is diverse and hybridization within and among species is possible. This affords geneticists great opportunities to capture heterosis and to combine desirable traits. However this same diversity causes variability among poplars in their ability to reproduce from cuttings. Hybrids may have desirable growth, health, or adaptability characteristics but if their cuttings do not root and survive in the field, they will not be used commercially.

The genus *Populus* is divided into several “Sections” which contain groups of species with similar characteristics. Poplars from Section *Populus* (the white poplars and aspens) reproduce vegetatively by suckering[2]. Field-planted cuttings of these taxa usually die because they produce above-ground shoots but do not develop supporting roots. Alternatively, poplars in Section *Aigeiros* (the cottonwoods and black poplars) can be easily propagated from cuttings. *P. deltoides* is the poplar from this Section that is native to the central United States. It is well adapted and roots moderately well, but breeders have both improved rooting success and growth by crossing *P. deltoides* with *P. nigra* (European black poplar). These interspecies hybrids, collectively known as the *euramericana* hybrids, are the most widely used poplars in the Lake States region (Dickmann and Kuzockina, 2014).

[1] In this context, a “steckling” is a cutting that has been allowed to develop shoots and root system in a greenhouse environment before being planted in the field.
[2] “Suckers” are shoots that develop from below-ground adventitious buds in the root system.

Rooting of cuttings is often encouraged through the application of auxin (a plant hormone that, among other things, can stimulate root growth). A group of diazotrophic microorganisms (endophytes) associated with poplars has been shown to produce auxins in culture (Xin, *et. al*. 2009) and to stimulate root and shoot development in *P. deltoides* (unpublished), *P. trichocarpa* and *P. trichocarpa* × *deltoides* hybrid cuttings (Knoth, *et. al*., 2014) when grown in greenhouses. Here we inoculated cuttings of three diverse poplar taxa with one of these auxin-producing endophytes, planted them in a field trial in northern Michigan, and monitored their vigor and height growth for 55 days.

METHODS

Three taxa of poplar hybrids were selected for field testing based on their expected rooting propensity. One representative from Section *Populus* was chosen. This was a hybrid of *P. alba* X *P. grandidentata* ("AG15" known here as "**AG**") developed by Pat McGovern (a private breeder from southern Michigan). This hybrid had shown superior growth and form in Michigan progeny trials. Previous plantations of this hybrid were established using stecklings because cuttings of this hybrid had never survived well when planted directly in the field.

The other taxa included here were from Section *Aigeiros*. They were developed by the breeding program of the University of Minnesota's Natural Resources Research Institute (NRRI). A single interspecies hybrid of *P. deltoides* X *P. nigra* (NRRI clone "99007115" known here as "**DN**") was chosen based on its superior growth in Michigan field trials. This hybrid was expected to root and grow well regardless of inoculation. Pure *P. deltoides* hybrids (known here as "**DD**") were represented by four NRRI hybrids ("9603-35", "9607-27", "9608-25", and "2801-06") that had shown good growth in limited Michigan field testing. It was necessary to use four DD hybrids to obtain enough cuttings for the field test. Cuttings of each DD hybrid were kept together by blocks (see the experimental design below). These DD hybrids were expected to root and grow moderately well but to improve as a result of endophyte inoculation.

A strain of *Rhodotorula graminis* endophytes isolated at the University of Washington ("WP1") was chosen after encouraging increases in above- and below-ground biomass were observed in developing *P. trichocarpa* cuttings in previous testing (Knoth, *et. al*., 2014). Half of all cuttings were inoculated by soaking them for 44 hours in a room temperature water solution of WP1 (optical density at 600nm of approximately 0.1) prior to planting. The remaining cuttings were soaked in pure water for the same time prior to planting. This latter group served as an uninoculated control. The bottom two-thirds of these 24 cm-long cuttings were submerged during the soaking.

An old-field site at Michigan State University's Forest Biomass Innovation Center in Escanaba, Michigan was selected for the test. The site was prepared by applying glyphosate herbicide to actively growing old-field vegetation in May, 2015. When existing vegetation was dead, the site was cultivated to a depth of 25cm. Soil at the site was a fine sandy loam from the Onaway soil series. Cuttings were planted on June 6, 2015 by fully inserting them into the freshly cultivated soil.

Cuttings were planted in a completely randomized block arrangement with four blocks. Each block contained six, 15-tree plots (one 15-tree plot of each inoculated taxa, and one 15-tree plot of each uninoculated taxa). Due to the shortage of cuttings, each block contained a single, but different DD hybrid. Vigor of the developing trees was scored every two weeks following planting and tree heights were measured after 55 days. During that time the site received 95 cm of rainfall and experienced 543 growing degree days (base 13º C using the Baskerville-Emin method). No weed control was applied during the 55-day trial to avoid any interaction between herbicides and cutting development.

RESULTS

All treatments initially produced vigorous shoots (Table 1). Early shoot development relies on energy reserves in the cutting. Vigor of AG hybrids rapidly declined as the energy reserves of the cutting were depleted and supporting roots failed to develop. Vigor of DD and DN hybrids gradually increased as roots formed. Vigor of the latter two taxa declined slightly toward the end of the 55 day trial. This was attributed to increasing soil moisture stress caused by weed ingrowth (recall that no weed control was administered) and decreasing rainfall. Endophyte inoculation had no statistical effect on vigor at any of the observation intervals.

All but one of the AG plants died and so this taxa was not included in the analysis of variance in height growth. Analysis of variance in height growth of live trees revealed that the difference between inoculated and uninoculated cuttings was significant (α=0.001) and the difference between DD and DN taxa was significant (α=0.000). Significant block effects were also observed (α=0.001). Since different DD hybrids were used in each block, block performance is confounded by both genetic and environmental influences. No conclusions should be drawn from these data about the relative performance of these four DD hybrids.

Table 1. Vigor 3 poplar hybrids emerging from dormant hardwood cuttings over a 55 day period after inoculation with endophytes.
(Vigor Scoring: 2=healthy, 1=wilted or yellowing leaves, 0=dead)

Endophyte Treatment	*Taxa*											
	AG				*DD*				*DN*			
	Days After Establishment											
	13	*26*	*40*	*55*	*13*	*26*	*40*	*55*	*13*	*26*	*40*	*55*
Inoculated	1.96	1.30	0.18	0.04	1.62	1.84	1.74	1.32	1.62	1.95	1.95	1.78
Uninoculated	1.21	1.21	0.07	0.00	1.07	1.75	1.89	1.40	1.51	1.84	1.95	1.79
Mean	1.59	1.26	0.13	0.02	1.35	1.80	1.82	1.36	1.57	1.90	1.95	1.79

(There are no significant differences between treatment means for vigor at any measurement interval)

The DN plants were 48% taller than the DD plants after 55 days (Table 2). This was not unexpected since this degree of heterosis is typical in interspecies hybrids. More significantly, inoculated plants of both DD and DN hybrids grew 14% taller than uninoculated plants of the same taxa. This was a highly significant improvement. A cursory observation of excavated cuttings at the end of the trial did not reveal any obvious difference in the formation of roots on cuttings of either of these hybrids.

All but one of the AG cuttings died during the course of the 55 day observation period and the survivor was exceptionally small and weak (Table 2). Inoculation had no benefit for this hybrid, which did not root under these conditions.

Table 2. Average plant height of 3 poplar hybrid clones on day 55 of an endophyte rooting promotion study in Escanaba, MI
(4 blocks of 15-tree plots in randomized block design)

Endophyte Treatment	*Taxa*		
	AG	*DD*	*DN*
	Height in cm		
Inoculated	14.0	28.0	41.3
Uninoculated	0.0	24.5	36.3

(Differences between treatments for each taxa AND differences among taxa within each treatment are significantly different at α=0.000)

CONCLUSIONS

The AG hybrid from Section *Populus* tested here did not benefit from inoculation with this strain of endophyte. There had previously been no success obtaining roots on cuttings of this hybrid under field conditions, and apparently the endophytes used here did not improve on this poor record.

Inoculated cuttings of both DD and DN hybrids grew 14% taller than uninoculated cuttings in the 55 days of this trial. This study ended without determining if this 55-day advantage in height growth will persist. If endophyte inoculation translates into long-term yield improvement, this would significantly improve the finances of poplar short rotation production systems. Increase yields without the addition of chemical fertilizers would also benefit the energy balance of poplar crop production.

Additional questions remain unanswered by this study. For example, it is not clear if the lack of response in the AG hybrid was because the endophytes had no effect or if the endophytes failed to successfully inoculate the cuttings. It is also not clear whether the improvement in DD and DN hybrid field height growth was due to an increase in rooting or some other factor, such as increased diazotrophic activity in inoculated cuttings. Finally, the extent of variability caused by environmental factors and poplar genetics as they interact with different endophyte consortia remains largely unexplored. Additional field testing will be required to unravel these questions.

LITERATURE

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