

Change of clonal frequency in the second root sucker generation of hybrid aspen



Lars-Göran Stener^{a,*}, Dainis Rungis^b, Viktorija Belevich^b, Johan Malm^a

^a The Forestry Research Institute of Sweden, Ekebo 2250, 268 90 Svalöv, Sweden

^b Genetic Resource Centre, Latvian State Forest Research Institute, "Silava", Rīgas 111, Salaspils LV-2169, Latvia

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ABSTRACT

Two hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) trials in southern Sweden were used for studies of clonal composition in the second of two root sucker regenerations. Trial 1 was established in 1998 and originally included eight clones randomly distributed in four plots, each having 10 × 10 positions. Trial 2 was planted in 1957, with 150 seedlings from each of 25 full sib families randomly planted. Genotyping, using six SSR markers, was performed on wood samples collected from the second root sucker generation in each trial.

Results from trial 1 clearly indicated a reduction in the genotypic diversity of the second sucker generation. All eight clones were still present, but at significantly different frequencies, with some close to zero. In trial 2, a total of 210 clones were found, genetically matched to 21 families, implying a rather marginal reduction in genetic variation. The clonal structure was mostly aggregated, but at the same time intermixing of ramets from different clones was quite common. The root suckers had, on average, spread 15 m from the stumps of the previous sucker generation of the same clone and the maximum distance was 49 m.

The results indicate a change in clone frequencies over generations in root sucker stands of hybrid aspen. Hybrid aspen reforestation with seedlings originating from several unrelated families will retain genetic variation better in future sucker generations compared to clonal reforestation in which few clones are used. It is appropriate to use a higher number of clones than the normal five to eight used in Sweden today, in order to prevent future clone-specific damage as a result of reduction of clone diversity after several root sucker generations.

1. Introduction

Interest in fast growing tree species has increased in Sweden in recent years. One reason is the urge to find alternatives to Norway spruce (*Picea abies* (L.) Karst.), which is the main planted species on fertile sites in Sweden, as a way to spread the risks in forestry of an uncertain future climate (Langvall, 2011; Kjaer et al., 2014). Another reason is the general aim to reduce Swedish dependence upon fossil energy (Anon.1, 2009). One strategy is to develop energy resources on forest and agricultural land, where intensive cultivation of broadleaved energy species is a feasible option. Sweden has large areas (ca 400,000 ha) of productive agricultural land available for growing biomass for biofuel (Larsson et al., 2008). Establishment of hybrid aspen plantations is one option on such land, as well as on suitable fertile forest land.

Hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) is currently one of the most interesting fast growing species for biomass production in northern Europe. Selected Swedish clones are estimated to yield approximately 25 m³ stem wood ha⁻¹ year⁻¹, during a rotation

time of 20–25 years on agricultural land in southern Sweden (Rytter and Stener, 2014). This corresponds to a dry weight of stem biomass of 8.2 tons DM ha⁻¹ year⁻¹ and to about 10 tons DM ha⁻¹ year⁻¹ if branch biomass is included. Similar growth has been reported in other north European studies (Tullus et al., 2012; Nielsen et al., 2014).

The ability to regenerate by root suckers after final felling is a great advantage in forestry involving hybrid aspen (Barring, 1988; Landhäusser et al., 2006). The number of root suckers is often at least 50,000 per hectare, of which many develop into independent individual trees after some years. Thus, suckering is a very useful and inexpensive method for the reestablishment of a new hybrid aspen generation.

The hybrid aspen material used today in Swedish plantations usually consists of a mixture of five to eight clones, planted at a 3 × 3 m spacing (1100 stems ha⁻¹). The fewer clones that are used in the mix, the higher the risk of an economic failure. For instance, if there is a serious pathogen outbreak during the rotation time, and some of the clones are damaged, the negative consequences will be reduced if the stand includes a wider variety of clones (Roberds and Bishir, 1997;

* Corresponding author.

E-mail addresses: lars-goran.stener@skogforsk.se (L.-G. Stener), dainis.rungis@silava.lv (D. Rungis), viktorija.belevica@silava.lv (V. Belevich), johan.malm@skogforsk.se (J. Malm).

Burdon, 2001). The significance of this is now important with regard to future climate change scenarios, which may result in new varieties of pathogens and other damage (Trumbore et al., 2015).

There are concerns about robustness with respect to clonal diversity in repeated generations of sucker stands. Therefore, it is necessary to characterise changes in clonal composition in the sucker regenerations of hybrid aspen in order to assess the potential impact of these changes on the viability of stands. For instance, will hybrid aspen clones with high growth capacity and high ability to produce root suckers out-compete other clones growing nearby. This may not be a problem, but if there are too few remaining clones, it may increase the risk of losses in yield due to clone-specific damage, as mentioned above.

The objective was to study the clonal composition in the second of two new hybrid aspen generations from suckers, by analysing the frequency of the clones in the second generation in relation to those planted originally. In addition, the clonal spatial distribution was analysed. The main hypothesis is that the most competitive clones are found at higher frequencies in repeated root sucker regenerations of hybrid aspen.

2. Material and methods

2.1. Material

Two hybrid aspen trials established by the Forestry Research Institute of Sweden (Skogforsk) in the southernmost part of Sweden were used. Finding appropriate stands for the study was not easy. The two stands selected, had different management regimes, were of different age and had no pedigree connections. However, they were both third generation hybrid stands created by root suckers after two final fellings, there were no other aspens in the nearby area and above all, we have knowledge of the reproductive material used. Annual mean temperature, mean precipitation (https://data.smhi.se/met/climate/time_series/year) and degree days (dd, based on daily mean temperatures $> 5^{\circ}\text{C}$, Perttu and Moren, 1995) are around $7\text{--}8^{\circ}\text{C}$, 600–700 mm and 1500 dd in the region where the two sites are located.

The first trial (Trial 1) is a yield trial in Snogeholm ($55^{\circ}33'\text{N}$, $13^{\circ}44'\text{E}$, 45 m), established in 1998 with the aim of studying the durability of root sucker regeneration after repeated final fellings. The trial is designed with 14 main plots, each including 10×10 plants, originally consisting of eight defined clones randomly distributed at a spacing of 3×3 m at the time of planting (Fig. 1). Each main plot was surrounded by at least two bordering rows of hybrid aspen, henceforth referred to as “border clones”, including a mixture of commercial clones and clones remaining from a propagation by Skogforsk, Ekebo. There is no clonal information available for the commercial material. However,

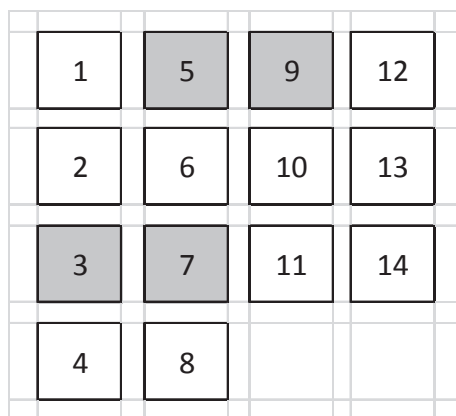


Fig. 1. General design of trial 1, Snogeholm. Each plot was 30×30 m surrounded by two border rows each, i.e. a total 12 m wide border zone. The plots marked in grey were used in the current study.

since Skogforsk was (and still is) responsible for the selection of commercial clones, we have knowledge of the material used at the end of 1990's. From this information we can conclude that there may be one clone (S21K8340001), that could have been planted both in the borders and in the main plots. All plots were completely harvested in the winter of 2008/2009 (age 11, generation 1) creating a dense stand of root suckers (Rytter and McCarthy, 2016). In winter 2012/2013 (age 4, generation 2) all root suckers in plots 3, 5, 7 and 9 and their surrounding border rows were clear cut, creating a second dense root sucker regeneration (generation 3), which was examined in this study. The other plots were managed in two other ways (strip cleaning) and were not included in the study.

The second trial (Trial 2) is located at Stehag, ($55^{\circ}54'\text{N}$, $13^{\circ}23'\text{E}$, 50 m) and was planted on fertile forest land in 1957. The material (Table 1) consisted of seedlings from 22 full sib families of hybrid aspen, two of indigenous aspen (*P. tremula* L.), one of *P. tremuloides* Michx. \times *P. grandidentata* Michx and, additionally, 11 families of larch (*Larix* \times *eurolepis* (Henry)). Each aspen family consisted of 150 seedlings, which were planted in a totally random design using a 1.75×1.75 m spacing. There were, in total, 3750 aspen and 700 larch seedlings planted in an area of 1.4 ha. The stand was divided into two sections (Fig. 2), which were thinned twice and the first final felling was carried out in the winters of 1985/1986 (part 1) and 1986/1987 (part 2). The new sucker regeneration, was gradually thinned forming a stand of 1500 trees per hectare (generation 2). Two commercial thinnings were performed and at the final harvesting of both parts in the winter of 2014/2015 there were 260 stems, ha^{-1} . A new dense regeneration of root suckers was then created during 2015, forming the third generation (Fig. 2). Some of the larch trees were kept at the first final felling, but they were all harvested at the 2014/2015 felling.

Wood samples from root sucker trees were collected from both trials in November 2015, i.e. at a sucker age of three years in trial 1 and one year in trial 2. For practical reasons, it was decided to use a systematic (quadratic) sampling design along parallel “lines”, based on compass direction.

The studied area in trial 1 was estimated to have around 80,000 stems ha^{-1} with a mean height of 5 m. The aim was to collect 150 samples in each of the four 30×30 m plots, giving an approximate distance between sampling points of 2.5 m. The sampling points were selected by pacing out this distance both along and between each compass “line”. Wood samples (10 cm long and 5–10 mm thick) were collected from branches or from the very top of the main stem, using a pole saw, and were taken from the tallest root sucker within a 0.3 m radius from the centre of each sampling point.

In trial 2 the aim was to sample a total of 1300 suckers covering the whole regeneration area (Fig. 2). The regeneration success was estimated to be at least 50,000 root suckers ha^{-1} in both sections. The height of the root suckers varied between 0.5 m and 2.0 m with an approximate mean of 1 m. The sampling methodology was the same as in trial 1 with some exceptions. The number of sampling points in each section was set in relation to their areas (6300 m^2 and 4400 m^2). Edges of around 10 m in width, close to nearby older stands of mixed broadleaved species, were excluded as were larger patches with few suckers, mainly found around harvested larches. A distance of 3.0 m was maintained between sampling points and a radius of 0.5 m from the sampling centre was used for selection of the sample trees. No samples were taken at points where root suckers within the 0.5 m radius were lacking.

Each sample was put into a separate plastic bag, marked with a unique number. The coordinates of each sampling point were registered by GPS (type Topcon GRS1) giving a position with a 0.2 m precision. Furthermore, in trial 2 wood samples were also collected for 205 stumps (diameter > 12 cm) from the last harvest (generation 2), and their coordinates and diameters were recorded. All samples were sent to the Latvian State Forest Research Institute “Silava”, Genetic Resource Centre, in Salaspils, Latvia for genotyping.

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