Forest Ecology and Management 356 (2015) 293–298

Contents lists available at ScienceDirect

Forest Ecology and Management

journal homepage: www.elsevier.com/locate/foreco

Pollen-mediated gene flow across fragmented clonal stands of hybrid eucalypts in an exotic environment

Paulo H.M. da Silva [a,*], Alexandre M. Sebbenn [b,c], Dario Grattapaglia [d,e]

[a] Instituto de Pesquisas e Estudos Florestais (IPEF), Avenida Pádua Dias 11, Caixa Postal 530, CEP 13400-970 Piracicaba, SP, Brazil
[b] Instituto Florestal de São Paulo, CP 1322, São Paulo, SP 01059-970, Brazil
[c] Faculdade de Engenharia de Ilha Solteira/UNESP, Caixa Postal 31, Ilha Solteira, SP 15385-000, Brazil
[d] Plant Genetics Laboratory, EMBRAPA Genetic Resources and Biotechnology, CEP 70770-970 Brasilia, DF, Brazil
[e] Graduate Program in Genomic Sciences Biotechnology and Universidade Católica de Brasília, SGAN Qd 916, CEP 70790-160 Brasília, DF, Brazil

ARTICLE INFO

Article history:
Received 15 July 2014
Received in revised form 2 December 2014
Accepted 4 December 2014
Available online 26 December 2014

Keywords:
Pollen flow
Paternity analysis
Outcrossing rate
Transgenic tree
Eucalyptus

ABSTRACT

We investigated the rate of pollen-mediated flow and realized reproductive success at increasing distances from the source, across fragmented clonal stands of hybrid eucalypts in Brazil by parentage analysis of grown out seedlings using genetic data at 15 microsatellite markers. Two study areas were employed: a pollen donor area composed of a clonal stand of a single pollen donor clone (PD) and a pollen sink (PS) area composed of a mixed clonal stand of two other clones (PS1 and PS2). In the pollen sink area four plots with 30 trees each, located at 25, 200, 400 and 550 m from the PD were established as sink islands. Before flowering, the entire clonal stands of clones PS1 and PS2, with exception of the sink islands were clear cut. Seeds were harvested from five randomly sampled trees in each sink island and the paternity of 15 seedlings per tree, 75 seedlings per island totaling a sample of 300 seedlings, was determined with PD as the alleged father. The self-pollination rate in the sink islands varied from zero to 24%. Paternity assignment to the PD was highest in the island at 25 m (17.3%) and rapidly decreased to 4.0% at 200 m and 2.7% at 550 m, suggesting a pattern of isolation by distance, while revealing a large pollen contribution from unaccounted sources across all islands. Our results in a fragmented clonal site agree with previous estimates of general pollen movement in eucalypt seed orchards, showing that pollination will take place by and large at relatively short distances of less than 200 m, consistent with the expected range of flight of pollinator bees. Nevertheless the exponential distribution observed also indicates that low levels of pollination success are to be expected over longer distances. Our data provide useful guidelines regarding the distance at which seed orchards should be established away from potentially large pollen pressure of clonal stands to minimize unwanted pollen introgression. Furthermore, our results on dispersal rate and distance of pollen have direct implications on gene containment strategies and modeling studies, as pollen-mediated gene flow is one of the key determinants of the potential ecological and biosafety impacts of prospective transgenic eucalypts.

1. Introduction

Understanding the patterns of pollen movement from planted eucalypt forests stands has important implications for nearby breeding operations and for considering the deployment of genetically modified (GM) eucalypts. Pollen dispersal data is vital to determine the distance at which seed orchards should be established away from commercial stands, germplasm banks or unimproved forests, so as to avoid undesired pollen contamination and its resulting negative impact on the genetic quality of the seed crops. Furthermore, by quantifying the distance of pollen flow from clonal stands, one may provide guidelines to contain or minimize gene flow from prospective transgenic eucalypts stands.

Although studies have assessed the potential seed-mediated gene flow from planted eucalypts into native forests in Australia (Barbour et al., 2010; Larcombe et al., 2013), US (Booth, 2012; Callaham, 2013) and Brazil (da Silva et al., 2011), little information exists regarding pollen-mediated gene flow across eucalypt stands in exotic conditions. Eucalypts are known to be pollinated by insect, birds and bats (Hingston et al., 2004; Southerton et al., 2004). In exotic conditions in Brazil, however, pollination is carried out largely if not exclusively by honey bees (*Apis mellifera*) which collect pollen from multiple trees and are expected to disperse it

* Corresponding author. Tel.: +55 19 210 58600.
E-mail address: paulohenrique@ipef.br (P.H.M. da Silva).

http://dx.doi.org/10.1016/j.foreco.2014.12.005
0378-1127/

at relatively short distances due to their limited flight range. Bee cages are ubiquitous in eucalypt forest plantations supporting an important honey industry (Leite et al., 2000). Pollen dispersal studies in *Eucalyptus saligna* plantations in Brazil using radioactive labeled pollen, revealed effective bee movement up to 100 m from their hives, with gradually decreasing activity up to a 300 m distance (Pacheco et al., 1986).

Pollen flow in eucalypts can be efficiently investigated using polymorphic co-dominant genetic markers that allow precise tracking of the paternal genetic contribution by analyzing marker allele inheritance to seedling offspring. The availability of well validated sets of microsatellites have made them the preferred working tool for this purpose in parentage studies in seed orchards (Chaix et al., 2003; Chaix et al., 2007; Chaix et al., 2010; Grosser et al., 2010), notwithstanding issues related to the occurrence of null alleles which need to be adequately addressed to avoid erroneous parentage assignments (Grattapaglia et al., 2004). Such parentage studies have shown complex and variable patterns of pollen dispersal within seed orchards depending on the genetic origin of the individuals involved, flowering synchrony and abundance, and overall ecological conditions. Invariably however, considerable proportions (29–46%) of pollen contamination of seed orchard crops from external sources have been reported, indicating that trees several hundred meters away from the orchard may ultimately contribute to seed production. Nevertheless no experiment to date specifically aimed at estimating the maximum distance of pollen flow in exotic environments, although an estimate of pollen flow distance of 1.94 km was reported for *Eucalyptus loxophleba* in natural stands in Australia (Sampson and Byrne, 2008).

The aim of this study was to carry out a systematic assessment of the rate of pollen flow and realized reproductive success at increasing distances between clonal stands of *Eucalyptus urophylla* × *Eucalyptus grandis* hybrids in tropical conditions in Brazil. Most industrially oriented breeding programs in Brazil make extensive use of outdoor controlled pollination orchards where elite trees are recombined to produce progeny on which selection is advanced. Although clonal deployment is generally preferred, open pollinated seed orchards are still used to provide high quality seeds for plantation in new areas where no clonal recommendation is yet available (Rezende et al., 2014). Furthermore, given the prospects of the impending deployment of transgenic eucalypts (Ledford, 2014) and the current standards of forest certification agencies that still do not allow commercial use of transgenic trees in certified areas, data about the rate of pollen flow across clonal stands is an indispensable information. We used a large set of well validated microsatellite markers and a maximum-likelihood framework for paternity analysis of seedlings to specifically try to answer the following questions: (1) What is the rate of pollen flow and realized reproductive success from a single pollen donor, assessed by genotyping grown out seedlings derived from seeds harvested in fragmented clonal stands of eucalypts? and (2) How does this rate of pollination success changes over increasing distances from the pollen donor source?

2. Material and methods

2.1. Study site and sampling design

The experimental area was established in a commercial clonal stand of *E. urophylla* × *E. grandis* hybrids, the most widely planted eucalypts in the tropics, at harvest age (6 yr). The experiment was located near Itapetininga city, Sao Paulo State, Brazil (23°35S, 48°03′W; and altitude of 670 m). To simulate a mainland to islands model of gene dispersion, we selected a clonal stand planted with a single clone, to function as pollen donor (PD) and represent the mainland (Fig. 1). A nearby mixed clonal stand established with two different clones was selected as pollen sink area with two clones (PS1 and PS2) representing the islands. Four islands were established at increasing distances (25, 200, 400 and 550 m) from the pollen donor clonal stand (Fig. 1). The 550 m distance was the maximum distance available at harvest age that would allow the experiment to be carried out. Although the experimental site was surrounded by commercial stands, we are assured that the specific clone used as pollen donor was not present in neighboring stands as well as in the four islands. In each island a square plot of 30 trees was sampled (6×5 trees = 180 m^2). Before flowering, the entire clonal stand of clones PS1 and PS2, with exception of the 30 tree square plots of the islands were clear-cut. After flowering and fruiting, open-pollinated seeds were collected from a sample of five randomly samples trees in each sink island, each tree therefore constituting a replicate. The number of trees of clones PS1 and PS2 varied across the islands (Fig. 1).

Seeds were geminated and grown at the nursery of the Instituto de Pesquisa e Estudos Florestais (IPEF). No difference was observed among the seedlings from different mother trees in terms of germination, morphology and initial survival. Fifteen randomly selected seedlings per replicate tree, 75 per island and 300 seedlings in total, were sampled at four weeks of age for the parentage study. Leaves from one PD tree and bark of all 20 clonal trees of PS1 and PS2 and leaves of the 300 seedlings were collected and subsequently used for DNA extraction, identity and parentage analysis.

2.2. DNA extraction and microsatellite genotyping

DNA was extracted from ~100 mg of fresh or cold air-dried leaf tissue using the extraction procedure optimized earlier for eucalypts (Grattapaglia and Sederoff, 1994). The three clones and the 300 seedlings samples were genotyped at 15 microsatellite markers (EMBRA2, EMBRA28, EMBRA3, EMBRA11, EMBRA10, EMBRA63, EMBRA157, EMBRA204, EMBRA219, EMBRA333, EMBRA128, EMBRA38, EMBRA210, EMBRA12 and EMBRA681), using PCR protocols and fluorescence-based multiplexed detection methods in an automatic genetic analyzer ABI3100XL as described earlier (Faria et al., 2010).

2.3. Diversity and parentage analysis

An initial assessment of the overall intensity of gene flow was carried out by looking at the basic genetic diversity measures in the sampled seedlings, both for each island individually as well as for all islands together. The following parameters were estimated: total number of alleles (k), number of private alleles (P_a), average number of alleles per locus (A), observed heterozygosity (H_o) and expected heterozygosity (H_e). The level of inbreeding was estimated using the fixation index (F) and the significance of the F values tested using 1000 Monte Carlo permutations of the alleles among individuals and a sequential Bonferroni correction for multiple comparisons (95%, $\alpha = 0.05$). All these analyses were carried out using Fstat (Goudet, 1995). Genetic relatedness among the three parental clones (PD, PS1 and PS2), was estimated by a pairwise kinship coefficient, using the Nason method (Loiselle et al., 1995), and implemented in Spagedi 1.3 (Hardy and Vekemans, 2002).

Paternity analysis was carried out under a maximum likelihood framework implemented in CERVUS 3.0 (Marshall et al., 1998), including the correction to accommodate genotyping inconsistencies (Kalinowski et al., 2007). To provide the critical LOD score values and the Delta (Δ) statistics (defined as the difference in LOD scores between the most likely candidate parent and the second most likely candidate parent) above which parentage would be assigned with a 95% confidence level, simulations were carried