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Maternal effects on phenotype, resistance and the structuring of fungal communities in *Eucalyptus grandis*

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ABSTRACT

The environmental experience of plants can modulate the development of the offspring and their interactions with other organisms. These effects, generally known as maternal effects, occur through seed provisioning and epigenetic modifications. This study considers the influence of differing environments of maternal plants on their progeny and their biotic interactions. Seeds were collected from two *Eucalyptus grandis* clonal seed orchards having different abiotic and biotic conditions. Seed and seedling development, and seedling responses to pest infestation and pathogen inoculation were measured. Finally, fungal communities in the foliage of the seedlings were assessed using a metabarcoding approach. The percentage of seed germination and height of seedlings were influenced by the maternal environments. Seedlings from one of the maternal environments were significantly more resistant to a pathogen than seedlings from the other. The composition and diversity of fungal communities also differed between the offspring from the two maternal environments. We found that the differences in the maternal environment affected the progeny performance and resistance. Moreover, we show for the first time that the maternal environment can influence the structure of fungal communities in the foliage in the subsequent generation.

1. Introduction

The environmental experience of plants can influence the phenotype and stress tolerance of their offspring (Agrawal et al., 1999; Holeski et al., 2012; Jablonka and Raz, 2009; Roach and Wulff, 1987). This transgenerational phenotypic plasticity, or parental effect, is not caused by modifications in the DNA sequence, but rather by the parental environment (Herman and Sultan, 2011; Roach and Wulff, 1987). In general, maternal plants are assumed to have a greater influence on the phenotype and resistance of their offspring than paternal plants. For instance, maternal plants directly provide seeds with substantial resources such as plastids, the seed endosperm and the seed tissues surrounding the embryo (Linkies et al., 2010; Rix et al., 2012).

The transmission of maternal effects to subsequent generations is not only related to seed provisioning, but also to epigenetic mechanisms. These epigenetic mechanisms are heritable transgenerational effects driven by reversible DNA methylation, histone modifications and small RNAs that alter the regulatory states of genomic regions (Hauser et al., 2011). In contrast, seed provisioning mechanisms refer to non-heritable transgenerational effects associated with carbohydrate, lipid, protein, and mineral nutrient reserves allocated to the seed by the maternal plant (Boyko and Kovalchuk, 2011; Herman and Sultan, 2011).

Maternal effects have been reported in many different plant species and traits. For example, differences in the seed traits of *Bromus madritensis* offspring can be the result of resource deficiencies due to competition in the maternal environment (Violle et al., 2009). In *Arabidopsis thaliana*, the maternal environment can affect the germination and flowering time of the progeny, as well as the seed mass produced by the progeny (Elwell et al., 2011). In other *Arabidopsis* studies, the progeny of plants inoculated with *Pseudomonas syringae* or exposed to caterpillar herbivory have been shown to increase resistance in comparison to progeny from unthreatened parent plants (Luna et al., 2012; Rasmann et al., 2012). For these reasons, maternal effects have been

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considered to be an adaptive strategy, offering an advantage to the progeny when it experiences similar conditions to the maternal environment (Galloway and Etterson, 2007; Mousseau and Fox, 1998). Consequently, a deeper understanding of these maternal effects could provide important predictions regarding, for example, stress tolerance of progeny based on the environmental experience of their maternal plants.

Seedling resistance against certain fungal pathogens varies depending on the maternal conditions (Vivas et al., 2013). This suggests that the association of the progeny with a broader fungal microbiome could vary depending on the maternal environment (Vivas et al., 2015). Such effects could be significant, given that microbial communities in the foliage of plants are thought to play crucial roles in the promotion of plant growth and protection (Peñuelas and Terradas, 2014; Vannier et al., 2015; Vorholt, 2012). Opportunities to study such maternal effects on plant microbiomes are increasing, because the in-depth characterization of microbial communities across a large number of samples is now feasible using high-throughput DNA sequencing (Rastogi et al., 2013). Such information could be used to understand plant ecology from the perspective of the extended genotype, which also includes the associated microbes and maternal effects.

To the best of our knowledge, no study has simultaneously tested biotic and abiotic maternal effects on seed traits, seedling performance and plant-pathogen \ plant-insect interactions in the associated plant progeny. Furthermore, nothing is known regarding maternal effects on fungal communities of these progeny populations. This study was consequently designed to consider the influence of maternal plants, naturally exposed to abiotic and biotic stressors, on their progeny and their biotic interactions. We examined the influence of maternal effects, different genetic backgrounds and their interactions using a *Eucalyptus grandis* W. Hill ex Maiden case study. *Eucalyptus* species are amongst the most widely utilized trees for the establishment of plantations globally, with major ecological and economic importance (Wingfield et al., 2015). Specifically, we considered these maternal effects and different genetic backgrounds on (i) seed and seedling performance, (ii) seedling resistance to a pest and a pathogen, and (iii) fungal communities in the foliage of seedlings (as proposed by Vivas et al. (2015)).

2. Materials and methods

2.1. Plant material

Eucalyptus grandis trees used in this study were located in two clonal seed orchards in South Africa, Greytown (29°11′56.73″S, 30°39′34.46″E) and Kwambonambi (28°35′41.45″S, 32°11′38.98″E). The orchards were planted in 2005 within a commercial tree breeding program and included a selection of 3rd generation *E. grandis* clones used for commercial seed production. The spatial design of the orchards was identical at both locations and, therefore, the pollen contribution in both orchards was expected to be the same. However, abiotic and biotic conditions differed among orchards. Kwambonambi orchard offered more conducive abiotic conditions for *E. grandis* growth than Greytown orchard. Specifically, Kwambonambi is situated in a low altitude subtropical region with higher rainfall and more effective tree root depth than Greytown, which is situated in a temperate region with lower rainfall and temperatures (Table 1). However, the Kwambonambi orchard was more heavily affected by pests and diseases as compared to Greytown orchard (Table 1). Three *Eucalyptus* genotypes (G1, G2 and G3) present in both seed orchards were selected. Each genotype was represented by three ramets in each orchard (2 maternal environments × 3 genotypes × 3 ramets). Ramet identity was confirmed and the possibility of pollen contamination discarded through DNA fingerprinting of six to seven seedlings per ramet (n = 120), using previously designed microsatellite markers and STRUCTURE analyses (Pritchard et al., 2000) (Methods S1).

Table 1
Characteristic of the *Eucalyptus grandis* orchards.

Conditions	Characteristics	Orchard	
		Greytown	Kwambonambi
Abiotic	Climate	Temperate	Sub-tropical
	Average Annual Temperature (°C)	17	21
	Minimum Annual Temperature (°C)	5	11
	Maximum Annual Temperature (°C)	25	29
	Average Annual Rainfall (mm)	832	1201
	Altitude (m)	1023	55
	Aspect	South	West
	Effective root depth (cm)	151	151 − 310
Biotic (pests)	*Leptocybe invasa*	Moderate	High
	Glycaspis brimblecombei	Absence- Low	Low-High
	Thaumastocoris peregrinus	Absence	Low
	Gonipterus scutellatus	High	High
	Phoracantha semipunctata	Absence	Absence
Biotic (pathogens)	Coniotyrium canker (*Teratosphaeria zuluense*)	Moderate	High
	Botryosphaeria canker and die-back (*Neofusicoccum* spp. and others in the Botryosphaeriaceae)	Moderate	High

Data provided by the forest company (Temperature and precipitation cover the years 1957–2007).

2.2. Experiment 1: maternal effects on seed and seedling performance

Seed capsules were collected from three ramets of each *Eucalyptus* genotype in each of the orchards. Seed mass was estimated as the total mass of seeds per capsule divided by the total number of seeds per capsule.

In July 2014, a factorial design of blocks was prepared with the seeds from the maternal environments and genotypes randomly distributed within each block. A total of 1620 pre-weighed seeds (2 maternal environments × 3 genotypes × 3 ramets × 90 seeds) were sown in Jiffypots® filled with vermiculite substrate in trays. The trays were placed in a common growth chamber with a 16 h day: 8 h night regime, 24 °C and 80% relative humidity, and watered as needed for 3 months. Individual seedling germination was assessed daily for two weeks and seedling height was measured weekly.

Seedlings were transplanted and moved to a common greenhouse in October 2014 and their height was measured once each month. At the end of the study, the diameters of the main stems of plants were measured directly above the root collars.

2.3. Experiment 2: maternal effects on seedling pest and pathogen response

Seedlings in the greenhouse were naturally infested (no forced infestation) with the Eucalyptus gall wasp *Leptocybe invasa* (Hymenoptera) in January 2015. *L. invasa* is one of the most damaging pests of *Eucalyptus* plantations outside its native range in Australia (Mendel et al., 2004). This wasp affects the new growth of all tree ages, causing galls and tree death in extreme cases (Dittrich-Schröder et al., 2012; Mendel et al., 2004). The number of galled leaves and the number of total leaves per plant was recorded to score the damage caused by *L. invasa* utilizing the rating scale of Dittrich-Schröder et al. (2012). In June 2015, all the seedlings were treated with a systemic insecticide (Imidacloprid 350 g l^{-1}) to prevent new infestations by *L. invasa*.

Isolate CMW2113 of the *Eucalyptus* canker pathogen *Chrysoporthe austroafricana* (Nakabonge et al., 2006; Wingfield et al., 2013) was selected to examine the influence of the different maternal