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Assessing Clonal Differences In Eucalyptus Seedling Resistance To Austropuccinia Psidii

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Abstract:
Austropuccinia psidii, commonly known as eucalyptus rust, poses one of the major threats to eucalyptus forest production and sustainability worldwide. This fungus pathogen infects a wide range of hosts, primarily species within the Myrtaceae family, causing significant damage to the forestry industry and ecosystem. In light of this scenario, evaluating the genetic resistance of eucalyptus plants to rust becomes a crucial component in management and prevention strategies. This study investigates the phenotypic resistance of different clones of Eucalyptus dunnii and Eucalyptus grandis to A. psidii, a rust pathogen. The experiments were conducted in the nursery area located in Irati - PR, Brazil. Nine clones, totaling 180 plants, were inoculated with a suspension of A. psidii urediniospores and evaluated for disease severity using a resistance scale. Results revealed varying degrees of resistance among the clones, with E. dunnii clones demonstrating resistance, while E. grandis clones exhibited susceptibility and high susceptibility. There were significant variations both within and between species. These findings underscore the importance of genetic variability within and between species in selecting resistant clones for planting and disease management strategies. Further research is needed to explore broader genetic pools and genotype-environment interactions to enhance breeding and selection outcomes.

Keywords: *forest pathology, myrtle rust, phenotyping, genetics*

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I. Introduction

Austropuccinia psidii (syn. = *Puccinia psidii*), commonly known as eucalyptus rust, is classified within the kingdom Fungi, phylum Basidiomycota, class Pucciniomycetes, order Pucciniales, family Sphaerophragmiaceae, genus *Austropuccinia*, species *A. psidii* (Beenken, 2017). The Pucciniales order is cosmopolitan, comprising over 7,800 cataloged species distributed worldwide, affecting a wide range of host plants (Ainsworth, 2008). These species cause diseases known as "rusts," a term derived from the wheat rust caused by Puccinia graminis f.sp. tritici Eriksson & Henning, with symptomatology presenting a color very similar to metal rust (Salazar & Carvalho Júnior, 2010).

A. psidii is considered to have originated in South America (Glen et al., 2007), first described infecting *P. guajava* (guava) in Brazil (Winter, 1884). *Austropuccinia psidii*, popularly known as eucalyptus rust or myrtle rust, poses a major threat to the production and sustainability of eucalyptus forests globally. This pathogen, a fungus, is responsible for infecting a broad range of hosts, mainly species within the Myrtaceae family, causing significant losses to the forestry industry and ecosystems.

In Brazil, Myrtaceae rust is among the most critical diseases for eucalyptus cultivation (Ferreira, 1989; Alfenaset al., 2009), causing severe damage to plantations (Coutinho et al., 1998; Pérez et al., 2011; Miranda et al., 2013). It poses a serious problem, especially when environmental conditions are favorable (Piza & Ribeiro, 1988).

Besides eucalyptus, *A. psidii* is pathogenic to various economically important botanical species of the Myrtaceae family, such as *E. stipitata* (araçá-boi), *P. cattleianum* (strawberry guava), *P. guajava* (guava), *S. jambos* (rose apple), *P. cauliflora* (jabuticaba), *E. uniflora* (Surinam cherry), *M. alternifolia* (tea tree), *P. edulis* (cambucá), *E. pyriformis* (uvaia), among over 90 other species documented as hosts of the fungus (Coutinho et al. 1998; Ferreira, 1989; Joffily, 1944; Rayachhetry et al. 2001; Silveira, 1951; Simpson et al. 2006).

The distribution of *A. psidii* encompasses the continents of Africa, Asia, Oceania, Central and North America, Caribbean and Pacific Islands, with various attacks recorded in the literature, citing countries like South

Africa, Germany, Argentina, Australia, Belgium, China, Colombia, Canada, Ecuador, the United States, France, Indonesia, Japan, Jamaica, New Caledonia, Puerto Rico, Paraguay, Portugal, the Dominican Republic, Uruguay, Trinidad and Tobago, Venezuela, among others (Coutinho et al., 1998; Carnegie et al., 2010; Giblin, 2013; Roux et al., 2013; Morin et al., 2014; Machado et al., 2015; McTaggart et al., 2016).

In Brazil, the pathogen has a wide geographical distribution, especially in the states of Bahia, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Rio de Janeiro, Paraná, Santa Catarina, São Paulo, and Rio Grande do Sul, potentially causing considerable damage depending on silvicultural management and the genetic materials used (Santos et al.,2001; Alfenas et al., 2009; Graça et al. 2013).

The parasitic cycle of rusts is didactically subdivided into five phases, being termed a macrocyclic rust (Coutinho et al., 1998), and when one of these phases does not occur, it is called microcyclic. According to Figueiredo et al., (2009), rust cycles are described as (0, spermogonium and spermatia; I, aecia and aeciospores; II, uredinia and urediniospores; III, telia and teliospores "probassidium"; IV, basidium "metabasidios" and basidiospores). Cycles occurring in a single host are called (autoecious) or if they occur through two phylogenetically distinct hosts (heteroecious). In the case of *A. psidii*, its life cycle is atypical compared to other rust species, being considered an autoecious and microcyclic rust with an incomplete cycle lacking the spermogonium (pycnium) stage and without an alternative host for this stage's occurrence (Morin et al., 2014). However, the possibility of the spermogonium phase occurring is not ruled out, but to date, it has not been demonstrated or described.

Infection is highly influenced by environmental conditions (Figueiredo et al., 2009) and occurs at the beginning of the host's sprouting, with the pathogen attacking young and tender tissues, such as leaves, inflorescences, and buds, being observed in both adult plants in the field and seedlings in the nursery phase (Ferreira, 1989; Figueiredo et al., 2009).

After infection, the symptomatology starts with chlorotic punctuations that transform into pustules containing bright yellow sporulation. These pustules may coalesce, covering the surface of the branch sprouts when the attack is intense. As a result, the affected tissues necrose, acquiring a black color, as if burned. After being infected, the plants can still form new leaves and branches after the pustules dry up, thus, the disease's incidence will result in reduced productivity in plantations and increased production costs when management measures are necessary (Furtado et al., 2009). In environmentally favorable areas for the disease, it presents itself as a limiting condition for the regeneration and development process of eucalyptus plants (Silva et al., 2013).

In Eucalyptus species, the control of *A. psidii* is mainly done by selecting and planting resistant genetic materials (Alfenas et al., 2009), in addition to planting susceptible materials at times and places unfavorable to the disease (aiming for escape).

The selection of resistant genotypes, along with integrated disease management practices, represents a sustainable approach to mitigate the impacts of rust in eucalyptus plantations. Therefore, the development and implementation of effective resistance assessment methods are essential for advancing silviculture and protecting the biodiversity associated with Myrtaceae species. This text aims to explore the main methods used in assessing genetic resistance to the *Austropuccinia psidii* pathogen, highlighting its importance and applicability in the context of eucalyptus production.

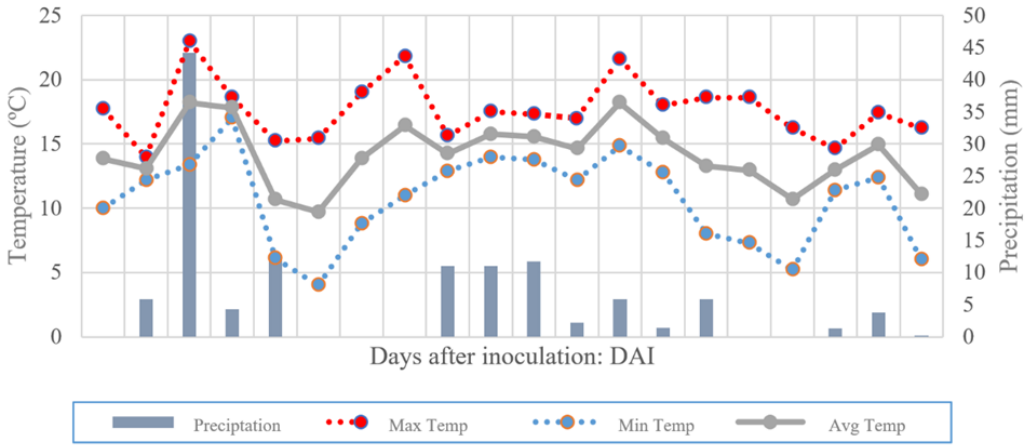
Given this scenario, assessing the genetic resistance of eucalyptus plants to rust becomes a crucial component in management and prevention strategies. The methods for assessing genetic resistance are fundamental to identifying and developing eucalyptus cultivars that can resist or tolerate *Austropuccinia psidii* infection, ensuring the productivity and longevity of plantations.

II. Material And Methods

Experimental area

The tests were carried out in the forest nursery area belonging to the Department of Forestry Engineering (DEF) of the State University of the Center-West (UNICENTRO) located at coordinates 25°32' south and 50°39' west in the municipality of Irati - PR. According to the Köppen climate classification, Irati's climate is type Cfb (temperate).

Figure 1: Graph of average, minimum, and maximum temperature vs. precipitation during the experiment duration (twenty days).



Source: INMET, Irati-PR.

Seedlings of nine clones of *Eucalyptus dunnii* and *Eucalyptus grandis* were used, with 20 plants for each clone, totaling 180 plants.

Table 1: List of clones and species for phenotypic evaluation of resistance to *Austropuccinia psidii*.

Clone	Specie
UNI0011	*E. dunnii*
UNI0012	*E. dunnii*
UNI0013	*E. grandis*
UNI0014	*E. dunnii*
UNI0015	*E. dunnii*
UNI0016	*E. dunnii*
UNI0017	*E. dunnii*
UNI0018	*E. grandis*
UNI0019	*E. grandis*

Source: Authors

Inoculations

The clones' plants were inoculated with a $1x10^4$ urediniospores/ml inoculum suspension using *Austropuccinia psidii* isolates from *Eucalyptus grandis* plants found in the Campos Gerais region. This suspension was evenly sprayed on both the abaxial and adaxial leaf surfaces using a backpack sprayer equipped with a fan nozzle producing 200 µm droplets, connected to a constant pressure valve of 2kgf/cm^2 on the spraying bar.

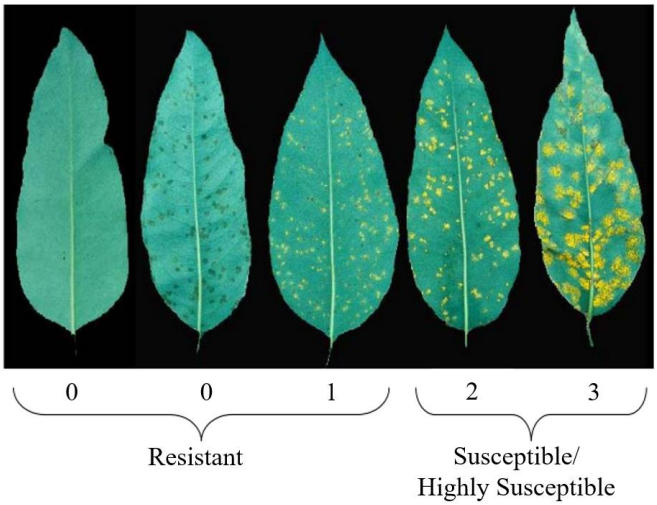
Post-inoculation, the seedlings were placed in a moist chamber, created by enclosing them with a transparent plastic bag (20 x 40 cm) secured with a rubber band, to ensure a high-humidity environment around the inoculum.

Disease severity was assessed 20 days after inoculation, using a modified version of the scale proposed by Junghans et al. (2003), which outlines four severity levels (Figure 2).

Severity Evaluation

The evaluation of disease severity was carried out 20 days after inoculation, adapting the rating scale recommended by Junghans et al. (2003), with four degrees of severity (Figure 2).

Figure 2: Scale for evaluating resistance to eucalyptus rust, featuring four severity classes: 0 = immunity or a hypersensitivity reaction of the "fleck" or necrotic type; 1 = pustules smaller than 0.8 mm in diameter; 2 = pustules ranging from 0.8 to 1.6 mm in diameter; and 3 = pustules larger than 1.6 mm in diameter (Junghans et al., 2003).



Source: Junghans et al., (2003)

In this study, we assessed the resistance of different *Eucalyptus* clones to a specific pathogen, 20 days post-inoculation (DPI). The clones were categorized into three groups based on their average symptom severity: resistant (severity < 2), susceptible (severity = 2), and highly susceptible (severity > 2).

III. Results And Discussion

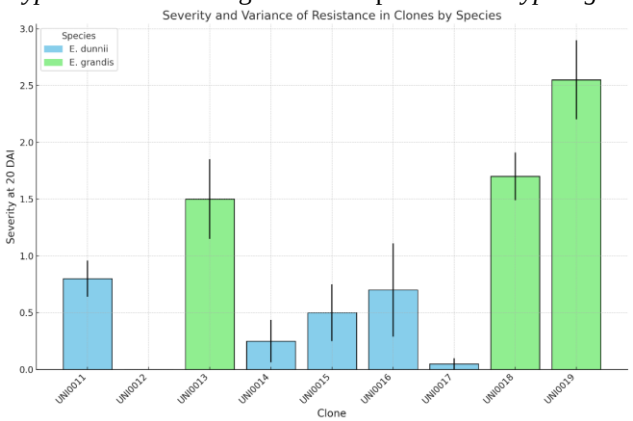
The results presented in Figure 3 illustrate the variation in the severity of rust (*Austropuccinia psidii*) among different clones of *Eucalyptus dunnii* and *Eucalyptus grandis*.

The clones UNI0011, UNI0012, UNI0014, UNI0015, UNI0016, and UNI0017, all belonging to the species *E. dunnii*, demonstrated resistance, with UNI0012 standing out for showing no disease symptoms (figure 3). This outcome suggests that the species *E. dunnii*, or at least the tested clones, may possess effective defense mechanisms against the pathogen in question, making them ideal candidates for planting in disease-prone areas.

Conversely, the clones UNI0013, UNI0018, and UNI0019, all from the species *E. grandis*, ranged from susceptible to highly susceptible, with UNI0019 displaying the highest average severity, indicating high susceptibility (figure 3). This implies that the species *E. grandis*, at least in the clones studied, might be less suitable for regions where the pathogen is prevalent, unless disease management measures are implemented to mitigate the risk. A possible alternative, depending on the conditions, could be chemical control.

The findings reveal a clear distinction between the two species regarding resistance to the studied pathogen. However, it's critical to note that the variance within the clones suggests individual variability in disease response, which could be leveraged in breeding programs to select resistant individuals within susceptible clones.

Figure 3: Chart of severity assessed 20 days after inoculation, using a rating scale, where the blue bars represent *Eucalyptus dunni* and the green bars represent *Eucalyptus grandis.*



Source: Authors

This study provides valuable insights for the agronomic management and breeding of Eucalyptus, emphasizing the importance of considering genetic variability within and between species when selecting clones for planting and disease management.

IV. Conclusion

The data highlight the importance of continuing research on the genetic resistance of eucalyptus to rust, considering the variability among clones and species. Future studies should expand the evaluated genetic pool and explore the genotype-environment interaction to maximize the effectiveness of selection and breeding strategies.

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