Identifying allelopathic compounds emitted by *Pittosporum undulatum* in Eucalypt forests

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Introduction

Climate change, habitat fragmentation and biotic invasion of exotic species are causing global biodiversity loss. *Pittosporum undulatum*, an aggressive invasive species affecting Australian plant communities, grows well in altered and degraded areas and its distribution has been increasing over the last decades.

Research aim

The aim of this study is to understand whether the invasiveness of *P. undulatum* is linked to the emission of allelopathic compounds from its leaves or if it is linked to the synthesis of beneficial compounds potentially improving its resistance to a changing climate.

Study area

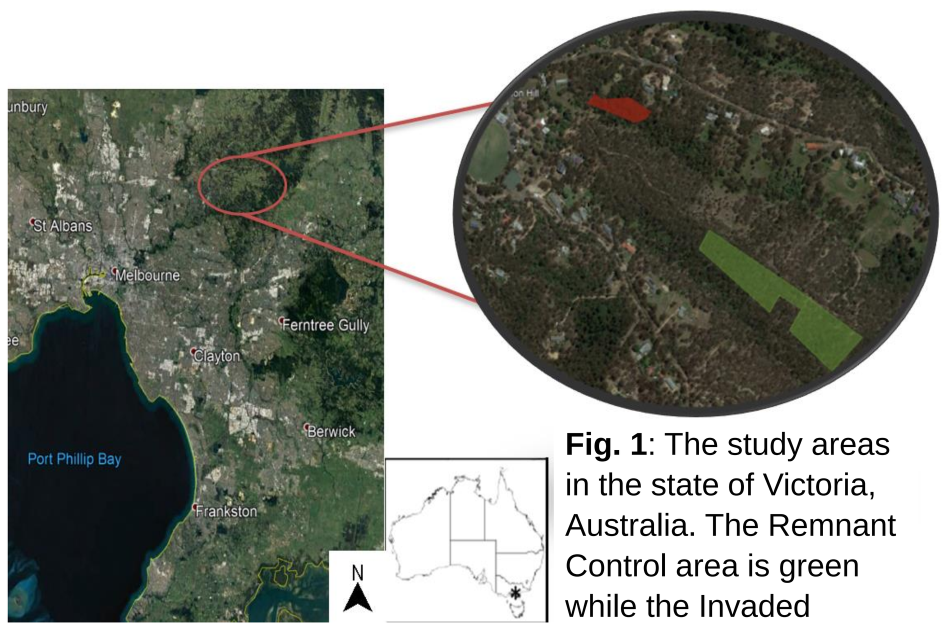

Fig. 1: The study areas in the state of Victoria, Australia. The Remnant Control area is green while the Invaded Control area is red.

The selected site is located in the north-east of peri-urban Melbourne area (Victoria, Australia) (Fig. 1). It is characterized by 2 different conditions: an area invaded by *P. undulatum* (Invaded Control) and an area with high quality native vegetation (Remnant Control).

Leaves were collected from both sites to produce extracts used in a germination test and then analysed to quantify their secondary metabolites content. Additionally, biogenic volatile organic compounds (BVOCs) were collected and analysed at environmental (plot) level.

Materials and methods

Germination experiment

A germination experiment was set up using:

- 3 different substrates: paper filter as control, sand and clay soil;
- 4 different types of watering treatments: distilled water as control, *P. undulatum* litter extract, *Eucalyptus spp.* litter extract and *P. undulatum* fresh leaves extract;
- 3 different species seeds: *Lactuca sativa* as control, *Eucalyptus ovata* and *P. undulatum.*

Chemical analyses

A BVOCs analysis was carried out at environmental level, using Solid Phase MicroExtraction (SPME) fibers.

Five fan samplers mounted with SPME fibers were installed in both areas (Fig. 2). Then, the fibers were desorbed directly in a GC-MS to identify the different compounds found in the air. Lastly, a characterisation of secondary metabolites from *P. undulatum* leaves and *Eucalyptus spp.* collected from the sites was performed. The leaves and the extracts from the germination experiment were analysed with HPLC.

Fig. 2: Volatile sampling with SPME fibers during the experiment in the Remnant Control area, Phantom Hill.

Results

No differences were found in seeds germination under different treatments (Fig. 3).

The forest invaded by *P. undulatum* shows a higher percentage of volatile monoterpenes, while natural *Eucalyptus spp*. forest has a higher percentage of volatile sesquiterpenes (Fig.4).

HPLC chromatograms (Fig. 5) showed that the extracts from *Eucalyptus* leaves present mainly derivates of ellagic acid, with some derivates of gallic acid and quercetin. On the other hand, extracts from *P. undulatum* leaves show a greater content of caffeic acid derivates and some derivates of luteolin and apigenin.

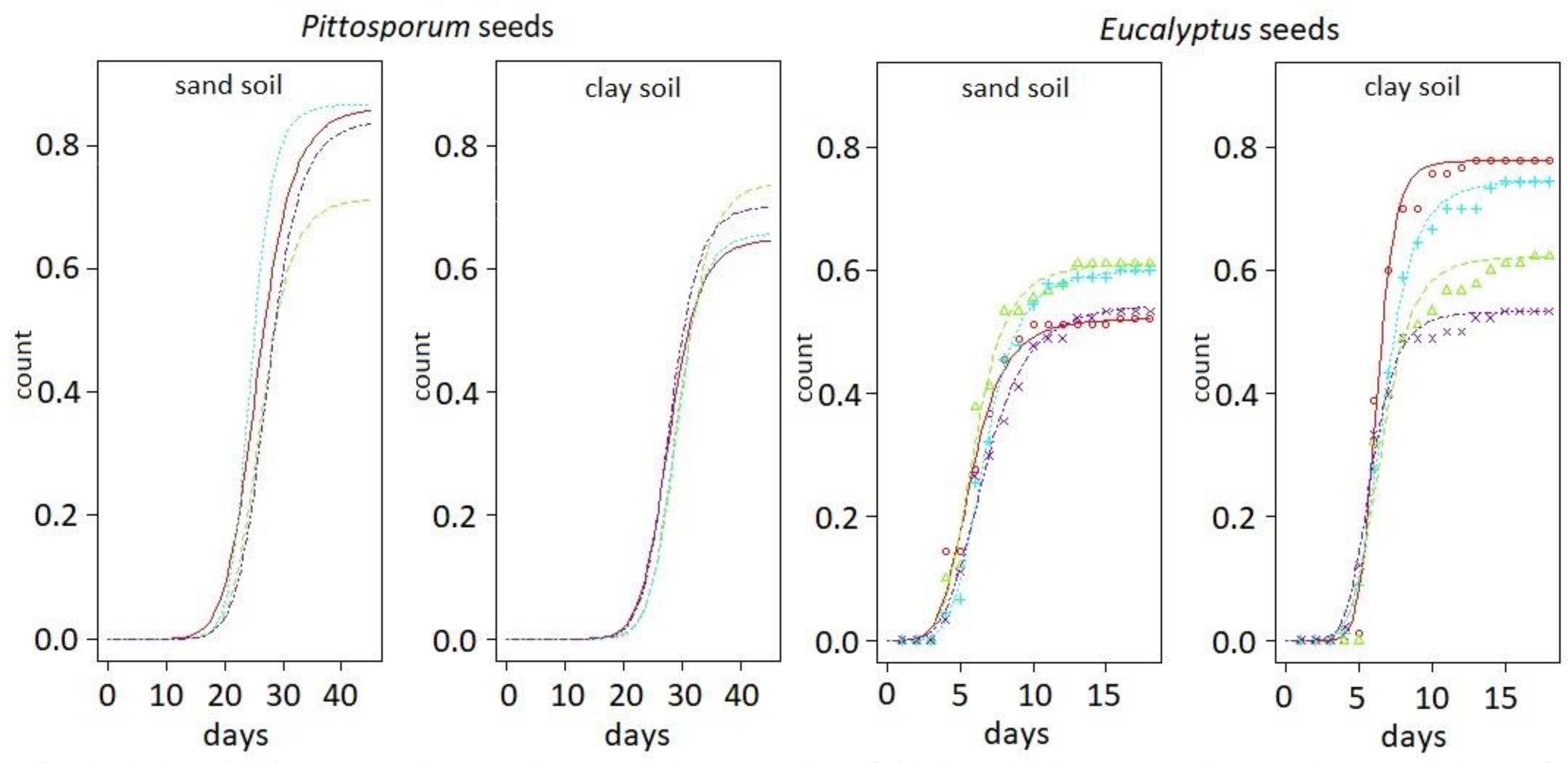

Fig. 3: a) Germination curves for *Eucalyptus* seeds on sandy soil; b) Germination curves for *Eucalyptus* seeds on clay soil; c) Germination curves for *Pittosporum* seeds on sandy soil; d) Germination curves for *Pittosporum* seeds on clay soil. The colors represent the watering treatments: red for water treatment; green for *Eucalyptus* leachate treatment; light blue for *Pittosporum undulatum* fresh treatment; purple for *P. undulatum* litter treatment. The x axis represents the time of the experiment and the y axis the percentage of germinated seedlings.

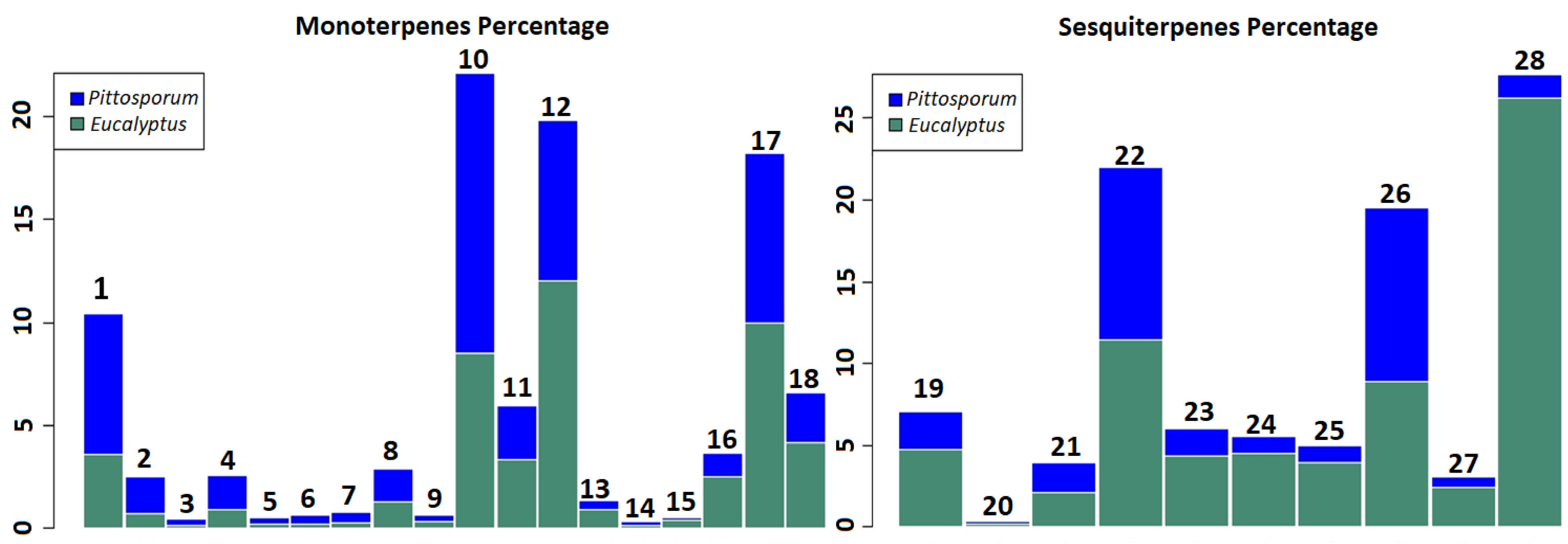

Fig. 4: Histograms showing the percentage of chemical compounds calculated on the total of each terpenic class. **1)** α–pinene, **2)** α-thujene, **3)** camphene, **4)** β-pinene, **5)** sabinene, **6)** car3ene, **7)** β-myrcene, **8)** α-phellandrene, **9)** α-terpinene, **10)** D-limonene, **11)** β-phellandrene, **12)** 1,8-cineole, **13)** o-cymene, **14)** terpinolene, **15)** allo-ocimene, **16)** δ-terpineol, **17)** α-terpineol, **18)** piperitone, **19)** α-cubebene, **20)** α-bergamotene, **21)** δ-gurjunene, **22)** aromadendrene, **23)** allo-aromadendrene, **24)** humulene, **25)** selinene, **26)** calamenene, **27)** globulol, **28)** δ-eudesmol

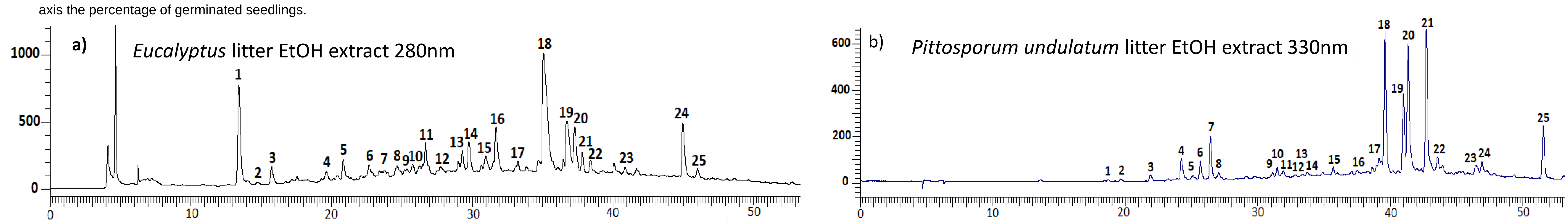

Fig. 5: Chromatograms obtained from HPLC: a) *Eucalyptus* extract in EtOH. The peaks 1,2 6 are derivates of gallic acid, the peaks 8, 10, 12, 15, 17, 21, 22, 23 are derivates of eucalyptin, the peak 13 is a derivate of vescalagin, the peaks 14, 16, 18, 19, 23, 24, 25 are derivates of ellagic acid, the peak 24 is derivate of quercetina. The others are unknown. b) *P. undulatum* litter extract in EtOH. The peaks: 1,2,3,4,5,6,7,8,9,10,11,12,13,14 are derivates of caffeic acid, the peaks 15, 16, 17, 22 are derivates of luteolin, the peaks 18, 19, 20, 21, 23, 24, 25 are derivates of dicaffeoylquinic acid.

Conclusion

Our results showed no differences in treatments during the germination experiment, suggesting that invasiveness of *P. undulatum* is not linked to the emission of allelopathic compounds. *P. undulatum* invasiveness is likely due to the biosynthesis of secondary metabolites that improve its resistance to abiotic stresses exacerbated by climate change. Indeed, both chemical analyses (GC-MS and HPLC) showed a higher amount of monoterpenes and caffeic acid derivatives in the forest invaded by *P. undulatum* than in the area with *Eucalyptus. Eucalyptus spp.* have mainly evolved defense mechanisms against mechanical damage rather than environmental constrains.

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