

Plant-herbivore interactions or colonization history: what drives changes in plant chemical defenses after invasion?

Introduction

Biological invasions offer a good opportunity to study rapid evolution of chemical defenses, because introduced plants encounter a complete novel biotic environment. Once in the novel range, plants may experience a decreased herbivore predation due to a loss of herbivores from the native area. This lower herbivory has been associated to changes in plant chemical defenses at population level. With no enemies associated, plants assigning more resources to growth and reproduction and fewer to chemical defenses would be favorably selected increasing the species invasion capacity, as suggested by the *Evolution of Increased Competitive Ability* (EICA) hypothesis. However, levels of secondary metabolites are highly determined by plant genetics, and thus observed changes in chemical defenses after invasion could also be a result of colonization history.

Here we determined the concentrations and profile of pyrrolizidine alkaloids (PAs) in *Senecio pterophorus* from its native, expanded and introduced ranges growing in a common garden design. Molecular neutral markers (AFLPs) were analyzed to determine populations origin. We discuss whether biogeographic differences in PAs are consistent to changes in plant-herbivore interactions, as predicted by the EICA hypothesis, or may be related to the plant invasion pathways.

Results (I)

PA concentrations. South African plants had lower concentrations of PA compared with plants from the two introduced regions. Plants from Australia showed the highest PA concentrations (Fig. 3).

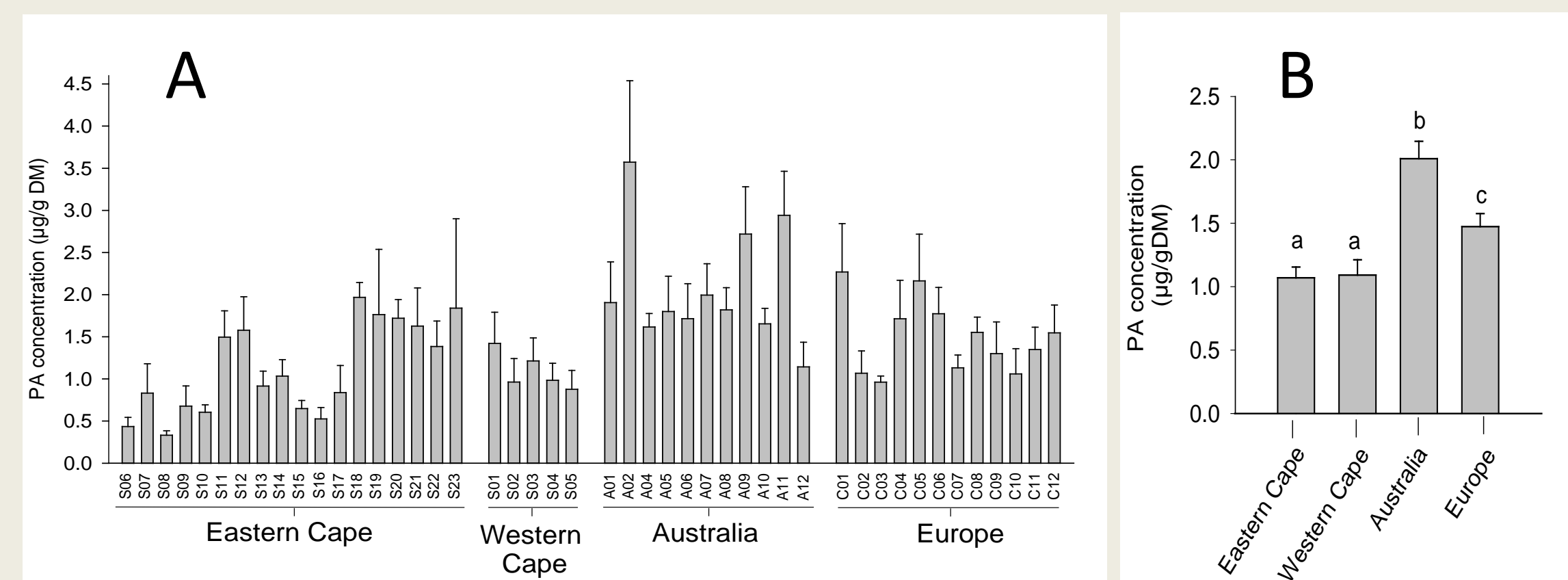


Figure 3. PA concentrations by populations (A) and regions (B). Different letters indicate significant differences in a GLM at $p < 0.05$

PA profile. Individuals from all regions contained senecionine, senecivernine and seneciphylline as the most abundant PAs. Plants from Australia and South Africa also contained rosmarinine-like compounds. Retrorsine was found only in South African plants (both Eastern and Western Cape). Cluster analysis showed that PA profile was homogeneous within European and Australian populations, respectively (Fig. 4). On the contrary, the chemical diversity within South Africa was very high. Individuals from the native Eastern Cape formed at least two distinct chemically-based clusters corresponding to different geographical areas.

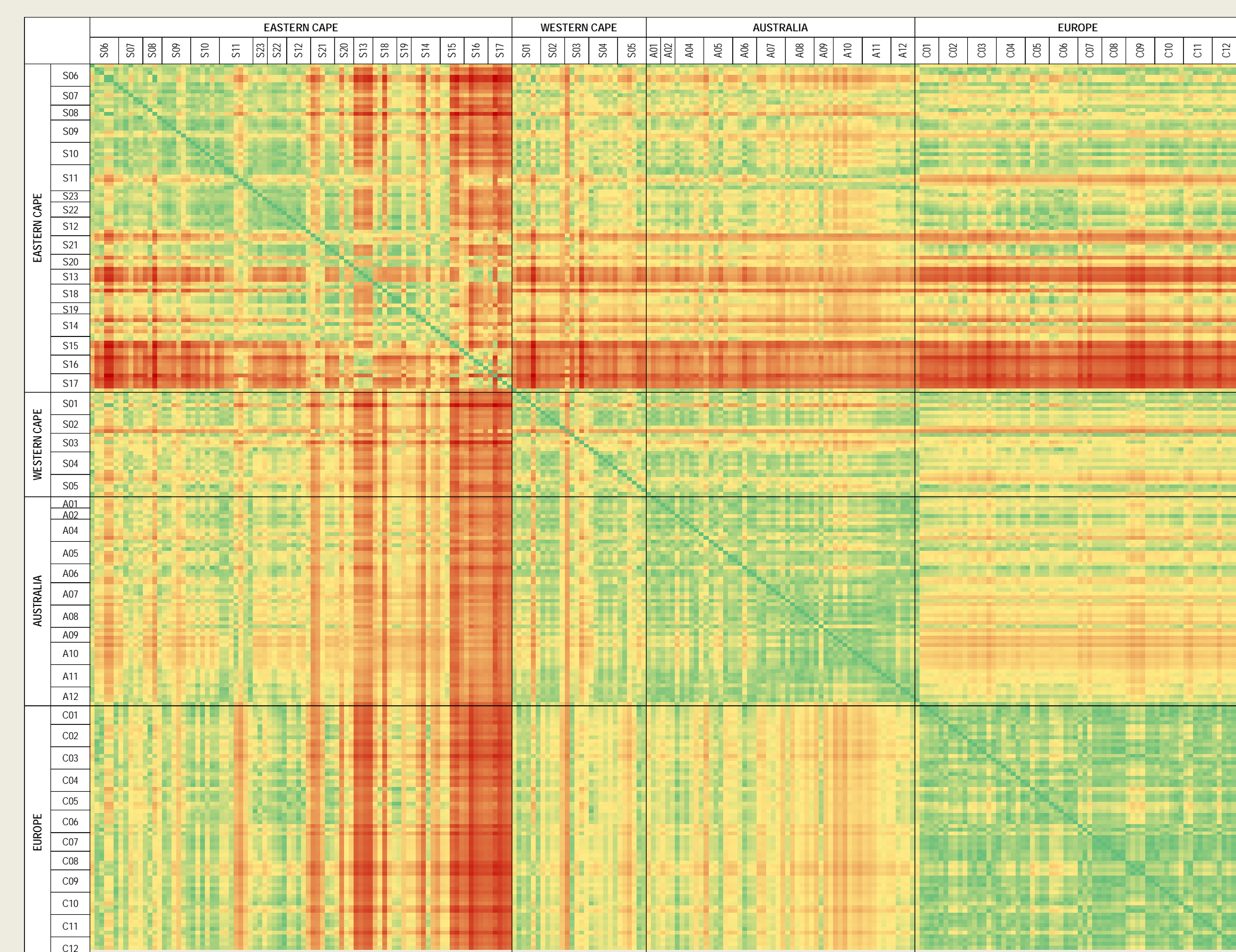


Figure 4. PA profile distance matrix of *S. pterophorus* individuals from South Africa, Australia and Europe. Color indicates similarity (maximum in green and minimum in red) between individuals in a hierarchical cluster analysis. Population numbers correspond to Fig. 1.

Model system.

Senecio pterophorus (Asteraceae) is a perennial shrub native from the Eastern Cape in South Africa, extended into the Western Cape, and introduced into Australia (> 70-100 years ago) and Europe (> 30 years ago). As other species of *Senecio*, *S. pterophorus* contains pyrrolizidine alkaloids (PA) that function as chemical defenses against herbivory. Worldwide distribution of *S. pterophorus* is relatively restricted (Fig. 1), which allowed us to conduct a broad biogeographical survey covering most of the areas where the plant was previously reported.

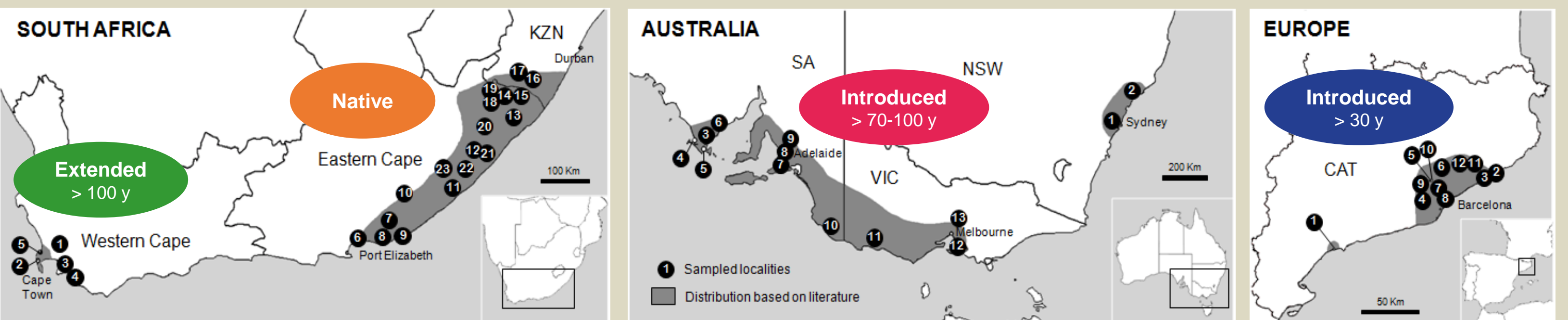


Figure 1. Distribution of *Senecio pterophorus* based on herbarium records and literature (shaded area) in the native (Eastern Cape in South Africa), extended (Western Cape, South Africa) and introduced areas (Australia and Europe). Leaves and seeds of 468 individuals from 48 populations were sampled for this study.

Methods

A biogeographical survey of 2-10 individuals from 48 populations was performed on the plant native, extended, and introduced ranges (Fig. 1). Leaves were used to estimate genetic similarities among populations by molecular neutral markers (AFLPs). Three EcoRI + 3 MseI + 3 selective primer combinations were used, and the presence or absence of each marker per individual plant was scored manually by visualizing electrophoregrams with GeneMarker v1.85 software (Softgenetic LLC). Seeds were used to conduct a greenhouse common garden experiment. Seedlings from each maternal plant were grown for ca 100 days (Fig. 2) and leaf PAs were analyzed by GC-FID (Agilent 6890). Total PA concentrations were estimated using monocrotaline as internal standard, and identification of individual PAs was carried out by a GC-MS. The percentage of each PA within individuals was calculated to determine the PA profile. A hierarchical clustering was then conducted using euclidean distances, and the resulting distance matrix was transformed into a heat map. Statistical analyses were conducted on SPSSv.19.

Results (II)

Phylogeography. Australian populations were originary from the Eastern Cape (Fig. 5), however population A12 was related to Western Cape populations (cluster G) but we could not discriminate the direction of this introduction.

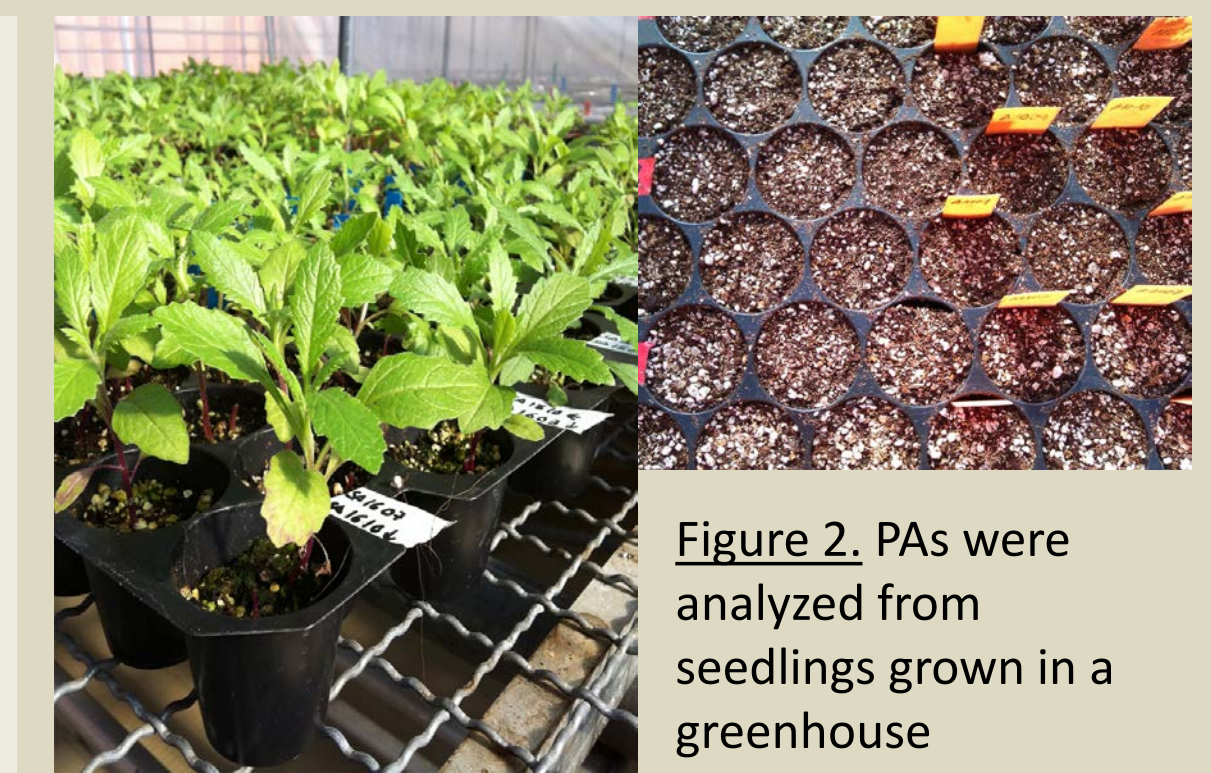


Figure 2. PAs were analyzed from seedlings grown in a greenhouse

European populations were a result of multiple introductions, mainly from South Africa (clusters D-F). However, population C01, which is relatively isolated geographically from the rest of European populations, was originary from the Australian population A01 (Cluster B). The origin of other European individuals could not be resolved (cluster C).

Discussion

In a previous study we found that *S. pterophorus* from the introduced populations suffered lower seed predation compared to those from the native populations. Moreover, this release was more pronounced in the region with the most recent introduction (Europe vs. Australia). Accordingly, under the EICA hypothesis, we would expect lower levels of PAs in plants from the introduced compared to the native ranges. Our results, however, are contrary to the EICA hypothesis because genetically-based PA concentrations were higher in plants from Australia and Europe compared to South Africa (Fig. 3).

Differences in PA concentrations were not fully explained by genetic similarities among populations, indicating that plant invasion pathways were not the main factor determining differences in PAs across regions. The PA profile was highly related to the population geographical distribution within region. However, chemical similarities between Australian and European populations with southern populations in the Eastern Cape (S06 to S20) in comparison to northern populations (S13 to S17) were not consistent with a closer genetic distance.

Conclusion. Introduced populations had higher levels and distinct PAs profiles compared to native populations, and these differences were not fully explained by the plant invasion pathways. Rapid evolution of PAs may have occurred after invasion, but the predation of herbivores in this process was not in accordance to the predictions by the EICA hypothesis.

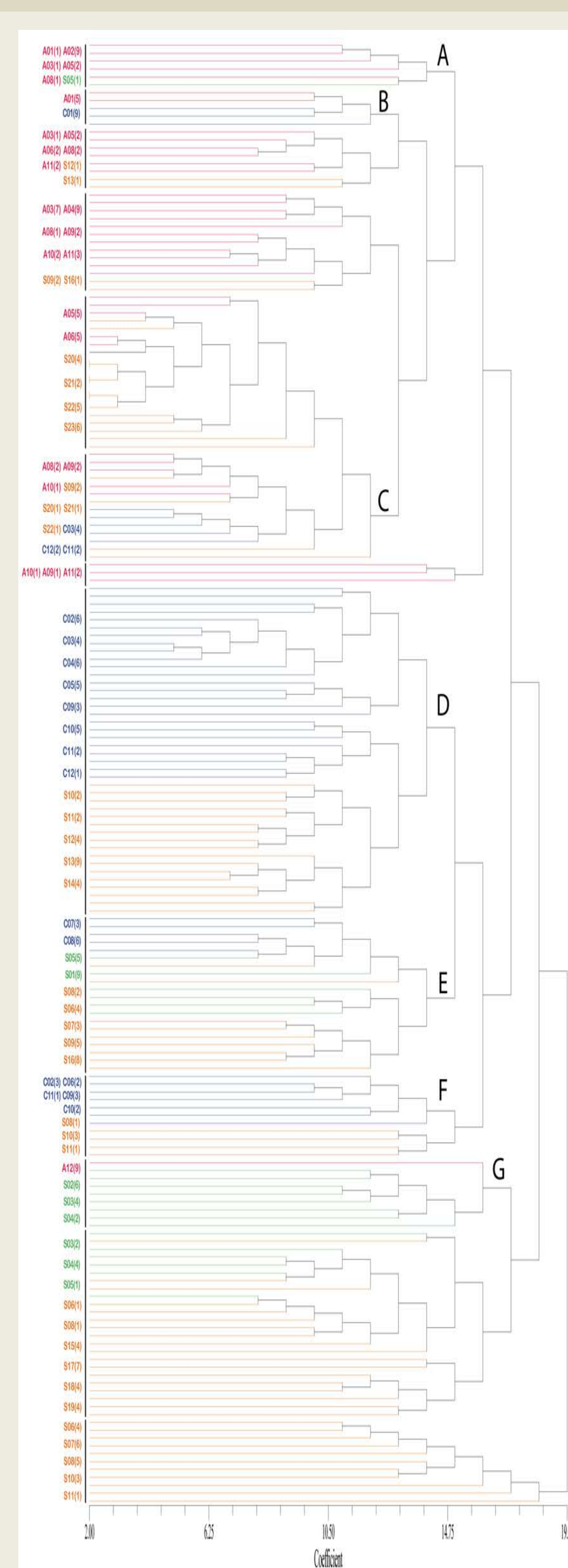


Figure 5. Pairwise genetic distances of AFLP phenotypes calculated by the Dice similarity algorithm using NTSyspc v2.02j (Applied Biostatistics Inc.). Colors indicate geographical distribution: Eastern Cape, Western Cape, Australia and Europe