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Proceeding Paper

Dynamics of Non-Structural Carbohydrates Reserves in Leaves of Two Perennial Woody Species, *Hakea sericea* and *Pinus pinaster* †

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Abstract: Non-structural carbohydrates (NSC) are key components of plant tissue, being involved in several metabolic and physiological processes, greatly affecting the growth and adaptation of plants. In this study, we compare the seasonal dynamics of leaf NSC, including total soluble sugars (SS) and starch (St), in *Pinus pinaster*, a tree native to the Western Mediterranean basin, and the invasive *Hakea sericea*, particularly problematic in areas with Mediterranean-type climate. With no exception, SS, St, and NSC contents changed throughout the growing season in both species, but with distinct patterns. The observed differences in NSC accumulation seemed to be related to the phenological cycle of both species but also suggest the superior performance of old *Hakea sericea* over *Pinus pinaster*, even under unfavorable environmental conditions, which may explain its invasion success in the Mediterranean region.

Keywords: soluble sugars; starch; seasonal variation; invasive plant species

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1. Introduction

Hakea sericea (Proteaceae) is a perennial shrub or small tree originating from south-eastern Australia [1]. It was introduced in several Mediterranean countries (e.g., New Zealand, South Africa, Portugal, France, and Spain), becoming a serious invasive alien species [1]. In Portugal, *Hakea sericea* is most troublesome in the northern part of the country due to the high occurrence of forest fires. It is often found in burned maritime pine (*Pinus pinaster*) stands, where it forms dense thickets.

A recent study evaluating the physiological performance of young plants (about four-year-old) of *Hakea sericea* and *Pinus pinaster* revealed that the invasive species possesses higher photosynthetic capacity than the maritime pine [2]. The superiority of *Hakea sericea* is also associated with higher contents of leaf photosynthetic pigments and soluble sugars, particularly in the summer season [2].

The accumulation of non-structural carbohydrates (NSC), composed by soluble sugars and starch, can be considered as an efficient growth strategy during periods of water restriction and high temperature, typical of summer months in Mediterranean climates [3]. Environmental stress such as drought generally induces NSC storage [4,5], building up reserves for survival when demands exceed supply [6]. The NSC content varies among sites, seasons, species, and tissues [7]. In general, NSC accumulation exhibits seasonal variability [8]. In addition, higher NSC contents are usually found in leaves and belowground reserve organs, as well as in herbaceous plants compared to conifers [6]. In deciduous plants, the accumulation of NSC occurs mainly in winter to support leaf formation in springtime [4]. In turn, in perennial plants, NSC storage occurs before dormancy [9,10]. It is generally assumed that storage of NSC may enhance competitive ability [7], which, therefore, may contribute to the success of invasive plant species [3,11]. However, the NSC dynamics of invasive plant species relative to native plant species are not very well understood.

In this study, we compared the seasonal variation in NSC, including total soluble sugars and starch, in current-year leaves of *Hakea sericea* with one native pine species (*Pinus pinaster*). The measurements were carried out from early autumn 2018 to early summer 2019 in old trees (approximately 50-year-old) of both plant species.

2. Material and Methods

2.1. Study Site and Climatic Conditions

The sampling site was located in the Alvão/Marão cordillera, north of Portugal (41°21′ N, 7°49′ W), which integrates the Natura 2000 network. This site is an intermediate zone between the Atlantic and Mediterranean climates. For a detailed description of climate, please see [2]. The area encompasses a large diversity of habitats, where oaks, mainly *Quercus robur* and *Quercus pyrenaica*; pine species, such as *Pinus pinaster* and *Pinus sylvestris*; eucalypts; and ericaceous and gorse shrubs are the most dominant species. Moreover, several patches of invasive alien species are also observed, including diverse *Acacia* species and *Hakea sericea*. *Hakea sericea* was introduced in the area at the beginning of the 20th century to form protective hedges. Its expansion has been associated with the increased occurrence of wildfires. Nowadays, the existing stands of *Hakea sericea* can be divided into long-invaded areas, where *Hakea sericea* persists for more than 50 years, and recently invaded areas, where the plants proliferated within the last 10 years.

2.2. Sampling Site and Data Collection

The sampling site was located in the south-western part of the SCI (41.18° N, 7.56° W), where the oldest plants (about 50-year-old) of *Hakea sericea* and *Pinus pinaster* were observed. Six plants of both species were marked and visited from autumn 2018 to summer 2019.

Leaf collection occurred in autumn, winter, spring, and summer. At each sampling time, 1-year-old leaves exposed to full sunlight and at the middle crown level were collected. To avoid irradiance effects and for consistency, all leaf collections were made between 12:00 and 14:00 h. The samples were placed in plastic bags for transportation to the laboratory under refrigerated conditions. There, the samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. They were subsequently lyophilized for one week at −55 °C and converted to a dried powder by grinding.

2.3. Biochemical Determinations

Total soluble sugars and starch concentration were determined by the anthrone method, using D-glucose as a standard. Total soluble sugars were extracted by heating the samples in 80% ethanol for 1h at 80 °C, and the methodology proposed by [12] was adopted for quantification. Starch was extracted from the same solid fraction by heating the samples in 30% perchloric acid for 1 h at 60 °C, according to [13].

Both biochemical analyses were conducted using a SPECTROstar Nano Microplate Reader (BMG LABTECH, Ortenberg, Germany).

2.4. Statistical Analysis

Seasonal and species effects were analyzed with a two-way analysis of variance (ANOVA), with six replicates. Comparisons among means were made using the Tukey test ($p < 0.005$). All analyses were run in IBM SPSS version 23 for Windows (Orchard Road-Armonk, NY, USA).

3. Results and Discussion

Total soluble sugars (SS), starch (St), and their sum (NSC) contents varied significantly during the experimental period (Table 1). Such seasonal changes have also been observed in other tree species of distinct climates, including tropical [3,7,8], Mediterranean [10], and boreal [14], and reflect the environmental conditions of the study area [7]. Regarding the seasonal course of SS, St, and NSC, our results revealed a distinct pattern of carbohydrate accumulation. In general, the average SS content was highest in early winter (50.8 mg g^{-1} dry matter), accounting for up 33-39% of that observed in the other seasons, and decreased about 39% throughout the year to a minimum of 31.1 mg g^{-1} dry matter in early autumn. Relative to the St content, the minimum values were found in early autumn (35.7 mg g^{-1} dry matter) and increased about 126% to a maximum of 80.7 mg g^{-1} dry matter in early spring. The NSC content was lowest in early autumn (66.9 mg g^{-1} dry matter), reached a maximum in spring (118.8 mg g^{-1} dry matter), and decreased significantly by 29% in summer. The observed SS, St, and NSC accumulation patterns agree with previous studies in other Mediterranean tree species [9].

Table 1. Leaf total Soluble Sugars (SS), Starch (St), and non-structural carbohydrates (NSC) of *Hakea sericea* and *Pinus pinaster* in each season (mean of six replicates ± S.D.). For each parameter, different lowercase letters indicate significant differences ($p < 0.05$) between seasons for *Hakea sericea*, and different capital letters indicate significant differences ($p < 0.05$) for *Pinus pinaster*.

Season	Species	SS (mg g^{-1} DW)	St (mg g^{-1} DW)	NSC (mg g^{-1} DW)
Autumn	*Hakea sericea*	34.766 ± 4.682 ab	34.969 ± 8.383 a	69.735 ± 11.601 a
	Pinus pinaster	27.539 ± 1.872 A	36.445 ± 3.552 A	63.985 ± 4.582 A
Winter	*Hakea sericea*	48.577 ± 3.818 c	59.883 ± 5.797 b	108.224 ± 6.337 b
	Pinus pinaster	53.029 ± 4.368 C	44.063 ± 2.568 B	97.092 ± 4.092 B
Spring	*Hakea sericea*	32.620 ± 2.572 a	103.603 ± 9.587 c	136.224 ± 10.787 c
	Pinus pinaster	43.655 ± 2.998 B	57.818 ± 4.553 C	101.472 ± 4.562 B
Summer	*Hakea sericea*	38.984 ± 2.567 b	36.247 ± 3.730 a	75.231 ± 3.344 a
	Pinus pinaster	29.384 ± 2.443 A	64.333 ± 6.445 C	93.718 ± 8.079 B
Two-way ANOVA				
Season (S)		<0.001	<0.001	<0.001
Species (Sp)		0.727	<0.001	<0.001
S × Sp		<0.001	<0.001	<0.001

Considerable seasonal variations in the contents of SS, St, and NSC were found between species (Season × Species interaction, Table 1). In both species, the highest SS content occurred in early winter, which corroborates the findings of [7]. This fact can be viewed as an adaptation strategy to cope with adverse weather conditions during winter. However, in summer, the invasive species presented higher SS content than the native, indicating that it is better able to withstand unfavorable warm summer conditions, and this can be viewed as an important feature for the resilience of the invasive plants [15]. In contrast to SS, the accumulation of St in *Hakea sericea* occurred in spring, while in *Pinus pinaster* it occurred during spring and summer. In addition, we also detected a distinct trend in both species: in *Hakea sericea*, the proportion of St increased from autumn to spring, whereas in *Pinus pinaster* the proportion increased from winter to summer. These fluctuations in ST and NSC contents can be explained by the phenological differences between the invasive and the native plant species. In *Hakea sericea*, the newly set fruit occur in early spring and mature in summer, while in *Pinus pinaster*, the fruiting development occurs between summer and autumn (personal observation).

4. Conclusions

Overall, our results indicate a clear seasonal variation in non-structural carbohydrate contents in *Hakea sericea* and *Pinus pinaster* that appears to be linked to the annual phenological cycle of both species. In addition, *Hakea sericea* exhibits higher accumulation of non-structural carbohydrates, especially soluble sugars, in summer, potentially representing a competitive advantage over the native under growth-limiting conditions typical of the Mediterranean climate.

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