PREFACE

The current presence of plant species in a particular area is explained by a series of events and processes of a diverse nature – mainly geological and climatic, but also anthropozoogenic – which have occurred over millions of years at different spatial levels. Sunlight is the energy source for plant photosynthesis and one of the major environmental factors influencing the growth and distribution of plant species. Most ferns live in moist and shady environments; however, some are able to recruit energy under high-irradiance conditions. Ferns are much utilized by people, namely for use as ornamental plants, as raw material to make handicrafts, in cosmetics, in foods and in popular medicine. This book discusses the biology, cultivation and implications for the environment of ferns, and also examines the Iberian Peninsula's shrub species.

Chapter 1 - The term Pteridophytes or ferns refer to vascular plants, but studies of the taxa can favor the distinction of the vascular plants on the basis of monophyletic lines, between the lycophytes and monilophytes (ferns). The ferns are plants with free seeds (spores), distinct vasculature with protoxylem confined to the lobes of the xylem strand, and these organisms reproduce by alternation of generations in two phases, the gametophyte and sporephyte. The pteridophytes occur in diverse ecosystems worldwide, from arctic-alpine regions to humid tropical forests. They occur throughout Brazil and are dependent on the high relative humidity, shade and mild temperatures, where this characteristic makes their life cycle dependent on available water in the environment for the germination of the spores and fecundation. However, ferns and lycophytes can also occur in semi-arid regions of Brazil known as the Caatinga and in the state of Minas Gerais, where the most abundant genus is Anemia. It is estimated that there are about 45,000 to 60,000 vascular

species in Brazil which represents 20% of the world's flora. Fossil evidence demonstrates the ecological success of vascular plants along the history of the earth, where records indicate that the pteridophytes possibly inhabited the earth from the end of the Devonian period and beginning of the Carboniferous period, and on the basis of modern cladistic analysis, there are ferns dating from the Upper Triassic period. Fossils of *Hopetedia praetermissa* were found in North Carolina (USA), and this species was confirmed as the oldest representative of the family Hymenophyllaceae. The pteridophytes are much utilized by humans, e.g., as ornamental plants, as primary matter for making handicrafts, as cosmetics, in foods and in popular medicine. Studies have analyzed parts of the fern *Dryopteris erythrosora* and shown strong bioactivity in the elimination of free radicals due to the presence of total flavonoids. Similarly, the pteridophytes *Abacopteris penangiana, Huperzia selago, Equisetum arvense,* and *Dryopteris crassirhizoma,* among other shave been shown to contain antioxidant components including flavan-4-ol glycosides, abacopterins, huperzine A, isoquercetin, and di-E-caffeoyl-mesotartaric acid, among others. Ferns studied in England of the families Pteridaceae, Sinopteridaceae, Vittariaceae, Dennstardtiaceae and others showed cyanogenic activity. The species *D. quercifolia*, *D. heterophyllum, D. lineares, B. orientale*, etc. of Western Ghats (India) showed bioactive agents with a wide antimicrobial spectrum. In other studies, also in India, in the Darjeeling district, extracts of *Cyclosorus interruptus* (Willd) H.I. *Gleichenia microphylla* R.Br, *Microsorium pteropus* (Bl.) and *Athyrium filix-femina* (L.) showed antibacterial potential. *Adiantum capillus veneris* was found to contain phytochemical components with both antibacterial and antifungal activity. Derivatives of chalcones have been found in the fern *Cyclosorus parasiticus* with the capacity to cause apoptosis in cancer cells as in studies by Tomšik. Besides the bioactivities cited above, pteridophytes can also have antiparasitic activity, in which a study demonstrated trypanocidal activity and antileishmania by parts of *Pityrogramma calomelanos* L. (Link). To extend their knowledge about *Pityrogramma calomelanos* L. (Link), the authors carried out cytoprotection tests using a heavy metal capable of producing free radicals, namely mercury chloride. This cytoprotection test was done with microorganisms, where the results were found to be satisfactory for *P. calomelanos*. It is believed that this result is due to the antioxidant components found in FRAP analysis, total phenols and flavonoids, and also to chelating activity. This is the first report of cytoprotective effects by part of a fern. The aim of this study was based on published studies indicating the use of *P. calomelanos* as well as other ferns in phytoremediation of soils contaminated

by metals. A cytoprotection assay demonstrated that *P. calomelanos* (L.) Link exerts an antagonistic effect in the presence of bacteria as well as fungi. Therefore, further evidence is presented of bioactivity of part of a pteridophyte, which warrants the isolation of active substances for better verification of this cytoprotective effect.

Chapter 2 - The Iberian Peninsula constitutes the south-western limit of the distribution range of several shrub species that are characteristic to European biogeographic regions different from the Mediterranean biogeographic region. This is particularly true along the north and west coasts of Spain for Atlantic species and in the Pyrenees Mountains for alpine species. But moreover, particular areas exist where Atlantic, alpine, and also boreal and continental shrub species occur at the edges of their geographical distributions. That is the case in the Iberian System: a vast area of mountain chains and massifs bordering the Inner Plateau of the Iberian Peninsula along its eastern side, and running northwest to southwest between Burgos and Valencia provinces in the autonomous regions of Castilla y León and Comunidad Valenciana, respectively. At its north-western extreme, peripheral populations of many shrub species occur, either isolated from conspecifics (disjunct populations) or in close proximity to other marginal populations. These populations show a high conservation value given their rareness and scarcity, for which they are being studied and characterised in order to implement appropriate management measures for their conservation. In this chapter the authors present several of these species, all of which are included in the regional Catalogue of Protected Flora of Castilla y León, placing an emphasis on the ecology and state of conservation of their peripheral populations in this area: *Chamaespartium delphinense* (a prostrated, suffruticose chamaephyte that inhabits the south of France in the Pyrenees Mountains, Dauphiné Alps and Vercors Massif), *Daphne cneorum* (Rock Daphne; a chamaephyte showing an Alpine distribution and present in the Pyrenees Mountains, and only locally at the eastern extreme of the Cantabrian Mountains), *Myrica gale* (Bog Myrtle; a deciduous nanophanerophyte characteristic of Atlantic European coasts, which is only locally present on the north-western Atlantic coast of Spain), and *Sambucus racemosa* (European red elder; a nanophanerophyte very widely distributed throughout boreal, Atlantic, alpine and continental biogeographic regions of Europe but whose nearest populations are found in the Pyrenees Mountains). Notably, all of these peripheral populations are: (1) very sensitive to habitat modifications, given that they are quantitatively scarce and their subpopulations spatially distant,

and (2) suitable indicators of changes in the climate, since they are found at the limits of their climate requirements.

Chapter 3 – Sunlight is the energy source for plant photosynthesis and one of the major environmental factors influencing the growth and distribution of plant species. Most ferns live in moist and shady environments; however, some are able to recruit energy under high-irradiance conditions.

Here, the authors tested 4 fern species and found that fern species adapted to high light always had a high photosynthetic rate. Shade-adapted ferns showed a low photosynthetic rate but high stomatal conductance at the initiation of light induction and required a short time to reach a maximal photosynthetic rate. In addition, when leaves were illuminated with 50 to 2000 $\mu mol\ m^{-2}\ s^{-1}$ photosynthetic photon flux density (PPFD) for 30 min, the degree of photoinhibition increased with increasing PPFD. Despite the different light-adaptation capabilities of the four species, they could be divided into 2 groups by degree of photoinhibition under the same PPFD.

Pyrrosia lingus, a slight-shade–adapted fern, could use more sunlight with high photosynthetic capacity, whereas *Asplenium antiquum*, a slight- to medium-shade fern, and *Diplazium donianum*, a medium- to heavy-shade fern, could dissipate excess light energy via xanthophyll-cycle–dependent energy quenching (qE).

Therefore, these 3 species could maintain low photoinhibition. These characteristics are probably related to their adaptation to direct sunlight (*P. lingus*) and sunflecks (*As. antiquum* and *D. donianum*). In contrast, *Archangiopteris somai,* a heavy-shade–adapted fern, showed both low photosynthetic rate and qE and therefore high excess energy and low photoprotection, for high photoinhibition.

Chapter 4 - Herbarium specimens are potentially valuable repositories of living spores and they may contribute for the preservation of fern diversity. This study aimed to evaluate fern spores preserved at the IICT Herbarium (LISC), in Lisbon, as regards of their capacity to germinate and to regenerate viable plants by using tissue culture techniques. Spores from herbarium specimens collected in Cape Verde [*Actiniopteris radiata* (Sw.) Link, *Anogramma leptophylla* (L.) Link, *Cheilanthes acrostica* (Balb.) Tod., *Cosentinia vellea* (Aitoh) Tod. – six and eight years of storage] and in São Tomé e Príncipe (*Cyathea welwitschii* Hook. - thirteen years of storage) were cultured *in vitro*. Only *A. leptophylla* eight years old spores and *C. welwitschii* spores failed to germinate. Culture media supplemented with different levels of sucrose and different concentrations of MS mineral salts were compared as

regards of their capacity to promote gametophyte growth and sporophyte development.

In *A. radiata* cultures, the best results concerning sporophyte production were obtained on media with 2% sucrose and 1/2 or 1/5 of MS mineral salts concentration. In *C. acrostica* and *C. vellea* cultures, best results were obtained on medium with 1% sucrose and 1/2 MS mineral salts concentration plus 150 mg/L NaH_2PO_4, while in the species *A. leptophylla* sporophytes were only produced on culture medium with 1/2 MS mineral salts concentration and 2% sucrose.