

## Bracken adaptation mechanisms and xenobiotic chemistry\*

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*Abstract:* As opposed to animals, plants have to cope with the resources, environmental restrictions, herbivores, and pathogens they find in the particular spot where they are bound to grow. Hence, resource sequestration, predation and competition relationships, and adaptation to various sources of other environmental stresses and their seasonal variation must be flexible enough to ensure survival and successful reproduction. Plants express this fitness by a combination of biological traits and chemical arsenals which operate under the reign of a genome of considerable plasticity. For the great majority of plants it is either the biological characters or the chemical composition that are explored independently to understand their fitness. But only in a few instances is the combination of these two avenues examined jointly. The extensive studies on the ecology, chemistry, and toxicology of bracken (*Pteridium aquilinum*) make this fern one of the few examples where a reasonable explanation for its extraordinary success is beginning to emerge by the combined perception of these two most important aspects of plant life. It is the purpose of this article to briefly review how the sum of biological and chemical traits cooperates to make of bracken one of the five most pernicious weeds in the world today.

### BRACKEN ENCROACHMENT

Bracken in its 12 different genets is a cosmopolitan heliophile fern growing in all five continents. Only the northern tundras, large deserts, and climatic forests are exempt of its great invasiveness. In the Americas, Chile is the only country where bracken is, curiously enough, not found. Possibly the barrier of the great Andean chain, the northern deserts, and the prevailing easterly winds from the Pacific Ocean block spore dispersal from existing stands in Bolivia and Argentina. The neotropical varieties of bracken, *caudatum* and *arachnoideum*, are altitudinally distributed to allow its growth from the sea level to the Andean periglacial zone, over 3200 m above sea level and well beyond the tree level. This is only possible if considerable genetic plasticity, residing in 104 chromosomes in the haploid genet, assists in providing the required adaptation mechanisms. *Pteridium* grows in dense thickets that frequently exclude other vegetation and cover vast expanses, sometimes with devastating economic consequences. Critical farming conditions due to bracken encroachment have been recognized in several countries including the British Isles, parts of Eastern Europe, New Zealand, Australia, various Asian countries, Central America, northern South America, Brazil, and Argentina. It not only grows ferociously but it is also intensely toxic to many vertebrates counting farm animals. In particular, cattle and sheep herds are deeply affected by the ingestion of this plant. Among the recorded diseases are severe disruption of blood cell counts, bone marrow degradation, and cancer of various internal organs. Animals usually do

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not recover and die [1]. Insects do not fare better since few species exploit the fronds as source of food and protection, and only in South Africa have been arthropods identified as bracken specialists [2]. Yet they do not imperil the survival of the plant.

The question arises as to what components of bracken complex natural life are responsible for this great ecological success. Chemistry undoubtedly must play a role, but not alone.

## BRACKEN SEXUAL AND ASEXUAL DOMINANCE

Bracken produces a prodigious number of microscopic spores, albeit variably from year to year, which may amount to 10 g per frond in the *arachnoideum* variety, or more than a billion particles. The spores are carried long distances by wind or animals that stray into the thickets. Although the germination rate of these spores is low, it is sufficient to colonize effectively new denuded territory [3]. The chemistry of spores remains unknown but toxicological evidence indicates that they contain xenobiotic material [4]. Bracken spores constitute a rare vacant niche, e.g., there are no known exploiters of this protein-rich resource [5].

Being a fern, *P. aquilinum* is a rhizomatose plant. This robust underground system, comprising up to 79.8% of the live biomass and  $5648 \pm 390$  kg/hectare (*arachnoideum*) [6], is not only long-lived, lasting perhaps several hundred years, but also provides various advantages over seed-based plants of the early succession. It stores nutrients that become readily available for new frond growth that develop quickly earlier than other seedlings. By piercing the soil deep underground the rhizome finds protection from wildfires. As a consequence, new blades sprout only days into the new succession at a fast pace to cover rapidly the available space. We have estimated the rate of elongation of rhizomes in a tropical habitat at 4.2 m/year, and a maximum blade growth at 56.7 kg/Ha/day on day 28 [7]. From the time croziers first emerge from the bare soil, around 17 days, to space saturation (100% area coverage) it takes less than 90 days. Blade maximum expansion is attained after only 35–45 days through four phenological stages. A fifth stage ensues in which tissues harden by accumulation of lignin and thickening of cell walls of chlorenchyma and sclerenchyma. Although fronds usually wither after three to five months of emergence, they remain standing, thus blocking light and forming a dense canopy, while rhizomes live for many decades. Hence, bracken displays advantageous traits both proper of pioneer species and of the old succession.

## Xenobiotic materials

In order to assess the role played by secondary metabolites in plant fitness it is essential to determine quantitatively the contents and variation of these compounds, either as chemical class or individually. For example, there are five phytoecdysteroids in *Pteridium* with powerful hormonal effects on insect ecdysis. However, these are present in lower quantity than required to cause any molting disruption in attacking arthropods – up to 53  $\mu\text{g}/\text{kg}$  fresh weight [8]. Radical changes in bracken's secondary chemistry occur during growth [9]. We have examined quantitatively the systematics of key xenobiotics—low molecular phenolics, polymeric tannins, prunasin, a cyanogenic glycoside, and illudanes—during frond development in neotropical *Pteridium* where insect pressure is high and there is no winter growth diapause. We observed that they appear to complement each other, perhaps under metabolic restriction and environmental necessity.

### *Phenolics and tannins*

Generally considered effective protectants against herbivory, flavonoids and phenolic acids among the low-molecular-weight derivatives and polymeric proanthocyanidins accumulate in bracken fronds as these grow. Values range from  $4.98 \pm 1.02$  mg of salicylic acid equivalents per g of biomass (gb) in stage I to  $21.27 \pm 4.62$  mg in stage IV for simple phenolics and  $6.68 \pm 3.0$  to  $32.38 \pm 9.20$  mg of tannic acid equivalents/gb, respectively. In places of high solar radiation such as the high tropical Andes, proan-

thiocyanidin storage may reach up to  $163 \pm 41$  mg/gb. That vacuoles in the parenchima and epidermal cells appear to lodge most of this material, and the highest concentration is found in exposed parts of the frond lead to the notion that these phenolics may also be involved in protection against abiotic pressures such as excessive UVB radiation. Bracken quercetin has been found to promote the transformation of mild virus-promoted papillomatosis, a relatively common disease in cattle, into severe carcinogenic lesions [10] whereas tannins are bacteriostatic, feeding deterrents and block protein digestion by precipitation. However, tannins alone in the observed quantities in bracken cannot stop feeding by all insects. For example, the leaves of the Andean tree *Alnus acuminata* hold up to 78 mg/gb of proanthocyanidins but are heavily depredated by certain oligophagous chrysomelid beetles that in turn ignore bracken growing at its feet. On the other hand, the phenolic acids and coumarin of bracken are released from rhizomes and decaying fronds into the soil to stall germination and growth of competing vegetation.

### Cyanogenesis

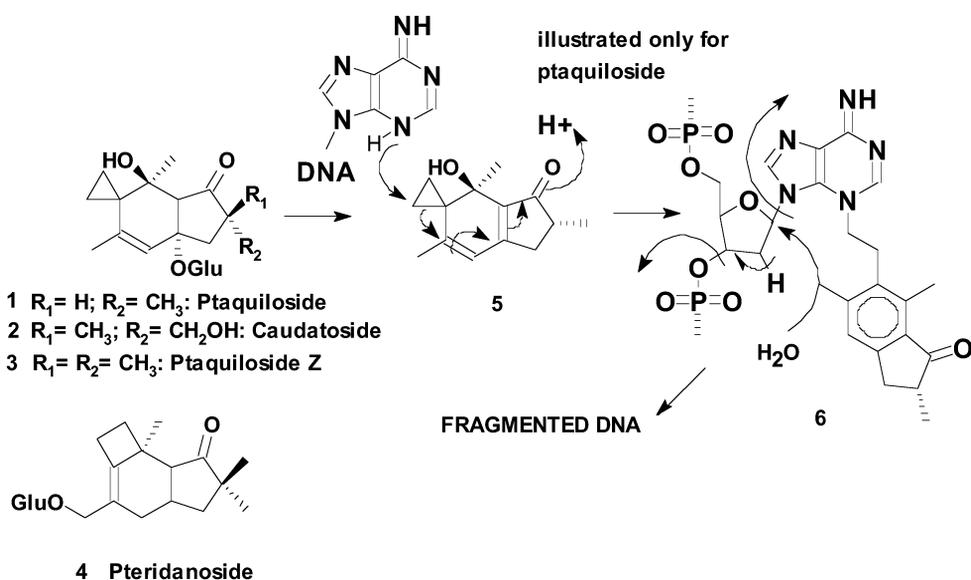
Only one HCN-producing material has been found in bracken: prunasin. Under the influence of prunasinase during cell rupture caused by animal feeding, HCN is released to deter many a predator. As opposed to phenolic material, the HCN production capacity decreases sharply along the frond phenological gradient. The rate of HCN evolution, a key factor in causing feeding deterrence, was found to be relatively slow yielding only  $2.03 \times 10^{-4} \pm 7.14 \times 10^{-5}$  mg [HCN] in a hypothetical 100-mg morsel of fresh frond devoured by an insect in 15 min. This amount of HCN is by itself not enough to cause direct feeding deterrence in arthropods. It represents only 0.01% of the prunasin present in the first stage or 5.84% of the second stage [11]. Hence, most of the cyanogenic glycoside must remain in the insect's gut and continue to release poisonous HCN, there causing additional harm to the attacker. Conversely, this nitrogen may be metabolized by adapted insects to their advantage. As a consequence, prunasin must be accompanied by other allelochemicals to effectively protect the growing tissue. These may be the illudanes.

### Illudanes

These are spirocyclopropyl sesquiterpene glycosides of powerful genotoxic properties. Ptaquiloside (**1**) was the first to be discovered but recently other illudanes (**2–4**) have been isolated from the Andean *caudatum* variety (Scheme 1) [12]. These compounds occur in larger quantity in the early stages of frond growth and decay rapidly thereafter, in parallel with cyanogenesis [13]. However, storage of illudanes varies also in inverse proportion with altitude. Transplants of rhizomes from high elevations in which ptaquiloside production is low to lower locations increase their ptaquiloside production to levels comparable to the surrounding bracken population after two years of establishment. Illudane dynamics are complicated additionally by other factors. For example, *caudatum* intermediate succession populations with a low yield of (**1**) increased 100 fold their illudane accumulation three months after fire had destroyed surface vegetation, possibly under the influence of nutrient input from ashes and alleviation of plant competition.

Structurally fragile, ptaquilosides decompose under mild base or moderate temperature to yield a highly electrophilic dienone (**5**) that is prone to attack by a variety of natural nucleophiles. Among these are the  $N_3$  of adenine bases of uncoiled DNA that form a covalent adduct (**6**). Adenine–dienone adducts have been shown to promote depurination by beta-elimination on the ribose nucleus and breackage of the phosphoribose polymer, leading to severe DNA disruption (Scheme 1) [14]. Such molecular alterations affecting key pro-oncogenes have been linked to various forms of cancer [15] which indeed are experimentally induced by feeding ptaquiloside to rodents and bovines, and are naturally observed in the field.

Substantial amounts of ptaquiloside have been found in milk from cows fed bracken fronds [16,17]. This implies that *Pteridium* not only deeply affects mammal predators but also their offspring through a multitrophic transport of its metabolites. Preliminary experiments also indicate that ptaquiloside is highly toxic to insects, suggesting that it contributes with prunasin to the protection of the vul-



Scheme 1

nerable early frond stages. Direct contact with bracken illudanes have been epidemiologically associated with gastric cancer in humans as well [18–20].

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