Influence of Defoliation and Environmental Constraints on Tannin Production in *Thamnochortus Insignis* Mast.

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ABSTRACT

The restio *Thamnochortus insignis* was investigated in order to establish the influence of defoliation and environmental constrainsts on secondary compound production. Plants were subjected to four treatments: fertilization, defoliation, defoliation and fertilization, shade. Results showed that defoliation reduced total polyphenolic content. No significant changes were observed in the shading and fertilization treatments. Culms of different ages (viz. new season's shoots, this season's reproductive culms and last season's reproductive culms) within the same plant ~~was~~ have analysed to determine if allocation to defense varied with age. It was found that this season's shoots had the lowest polyphenolic concetrations, followed by last season's reproductive culms, and this season's reproductive material showed the highest polyphenolic concentrations.

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INTRODUCTION

A knowledge of the chemicals produced by different plant species has probably been important since the early stages of man's agricultural development. A plant's chemical constituents are complex mixtures of many different compounds, the majority of which belong to the so called "secondary products" (Swain 1977).

The term "secondary plant metabolites" was first introduced by Kossel in 1891 (Baas 1990). They were so named because it was thought that they had no obvious metabolic function and served merely as waste products. At around the same time Stahl suggested that some of the chemical properties of plants may have evolved for protection against attack by herbivorous animals (Rhoades 1979). The idea lay dormant for a long while and it was only in 1959 that Fraenkel put together a comprehensive statement regarding the role of insects in the evolution of plant secondary compounds. Fraenkel suggested that as herbivorous insects have similar dietary requirements, the observed feeding patterns must be due to the presence of secondary plant compounds. Although his theories received much criticism, they laid lay the grounds for a new field of research. In subsequent years investigations into this field have grown and various theories on

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secondary compounds have arisen (e.g. Rhoades 1979, Coley et al, 1985, Swain 1977, Mooney 1972).

It is now widely accepted that plant secondary compounds are present to maintain the overall integrity of the plant under natural conditions. They may function as defense chemicals or, more generally, are involved in plant-environment interactions (Baas 1991). For the purpose of this study the terms "defense compounds" and "secondary compounds" will be used interchangeably.

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Plants have evolved an extraordinary chemical diversity of which over 10 000 low-molecular weight metabolites are known (Swain 1977). Secondary compounds can be broadly divided into 2 groups, the accumulating (quantitative) compounds and the minor (qualitative) compounds or toxins (Baas 1991). The quantitative group are high energy compounds which are costly to produce and accumulate in the plant. Polyphenolics such as lignins and tannins belong in this class, and are considered as biologically inactive (i.e. no costs involved in turnover).The qualitative compounds on the other hand are biologically active at much lower concentrations and include defenses such as alkaloids, phenolic glycosides and cyanogenic glycosides (Coley et al. 1985).

Quantitative defenses are so designed that they reduce the digestibility of plant tissues (Rhoades 1979). They are utilized extensively by both herbaceous and woody plants. Tannins are regarded as one of the most important groups(Swain 1977) of the quantitative defenses. Based on chemical structures these are divided into the hydrolysable and the condensed tannins. The former consist of a sugar core, usually glucose, the hydroxyl groups of which are acylated by gallic acid or its congeners (Bate-Smith 1973); while the latter are flavanoid polymers, with carbon-carbon bonds which link the flavanoid monomers (Bate-Smith 1973). Hydrolyzable tannins can be readily hydrolyzed into their component parts while condensed tannins are not susceptible to hydrolysis (Hagerman and Butler 1989). Both condensed and hydrolysable tannins interact with proteins to form either soluble or insoluble complexes. Their potential biological activity is thought to be related to their degree of protein precipitation (Bernays et al. 1989). It has however been demonstrated , by Asquith and Butler (1985), that the tannin-protein interactions are specific with respect to the different chemical structures, thereby implying different biological activities. Cooper and Owen Smith (1985) suggest that the condensed tannins' main role is to inhibit the microbial breakdown of plant cell walls. This will then affect herbivores which depend on microbial fermentation in aiding digestion of plant cellulose in the gut. In this manner the plant reduces the nutritional value of its material to ungulates. The astringent taste of certain plants is associated with the hydrolyzable tannins which act to inhibit the digestive enzyme activity in herbivores.

Bate-Smith (1974) suggested that condensed tannins probably evolved in the Carboniferous when their presence became widespread and that as Angiosperms evolved they first elaborated the hydrolysable tannins after which families evolved which had neither. The hydrolysable tannins occur only in dicotyledons while the condensed are found in most classes of plants (Bernays et al. 1989).

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"Because there are tradeoffs between a plant's various functions, the concept of costs and benefits helps explain allocation patterns at both the physiological and evolutionary levels" (Bazzaz et al. 1987).
The idea that an individual's primary functions (e.g. growth, reproduction, defence) compete for limited resources is now widely accepted (Bazzaz et al 1987). Costs and benefits can be considered as the way plants differ in their commitment to defenses, with respect to the various factors that govern their overall resource allocation processes. As plants differ in the way that they carry out their essential functions, the fact that they exhibit varying modes of defense (both between and within different plants) stands to reason.

With reference to individual plants; the optimal defense theory (ODT) predicts that organisms evolve and allocate defenses in the way that maximizes individual inclusive fitness (Rhoades 1979). Thus plants allocate defenses in direct proportion to their risk from predation and in inverse proportion to the costs involved. It would there fore be expected that plants not experiencing a high degree of predation, would allocate less of their resources to defense and consequently have a higher fitness. This is in turn reversed when predation is high. Furthermore within a plant defenses are allocated according to the risk and value of losing a particular tissue or organ (in terms of fitness lost to the organism) if that tissue or organ is subject to attack.

An alternative approach is that passive effects governed by abiotic factors may be more important than herbivores in determining plant allocation to defense. The "growth rate theory" predicts that both the nature and quantity of plant defenses are determined by the resources available in the local habitat (Coley et al. 1985; Coley 1988). Over evolutionary time plants have adjusted their inherent growth rates in accordance to available habitat resources. Resource limitation has resulted in a suite of interdependent characteristics associated with slow growth rates (Bryant et al. 1988).

teleology

Slow growing species have long-lived leaves (Coley et al, 1985) as slow leaf turnover is advantageous in a low nutrient environment. This is due to the large proportion of nutrients lost from the plant every time a leaf is shed. These plants therefore benefit by employing quantitative defenses which, although expensive to produce, require very little energy in terms of maintenance costs.

In contrast plants growing in resource rich habitats tend to exhibit rapid growth rates which involves a rapid leaf turnover. The nutrients lost when leaves are shed can be quickly replaced by the new leaves as resources are not limiting. Fast growing species with relatively short leaf life times appear to favour qualitative defenses (Coley et al, 1985; Coley 1988). This is largely attributed to the continual metabolic costs required over the entire leaf lifetime.

The "growth rate theory" then extends itself by predicting that species with slow growth rates should have high concentrations of defense in comparison to those with fast growth rates. Any environmental condition which selects for plants with inherently slow growth rates will therefore directly influence defence concentrations. Areas where water, light or nutrients are limiting have been reported to select for well defended plants (Baas 1989). Experimental evidence supports this

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as studies carried out by shading plants often result in a decrease of secondary compounds (Bryant et al. 1988).This can be explained as the insufficient light limiting photosynthesis thereby decreasing soluble carbohydrate synthesis, resulting in less carbon available for defense compounds. This not experimental evidence supporting the concept.

Studies of defense allocation in the Restionaceae and the grazing pressure that they may be subjected to, have to date, not been studied (Linder 1991). The flavonoid chemistry of the Restionaceae ~~have~~ has been investigated by Harborne (1979) and Harborne et al. (1985) revealing significant differences in the flavonoid constituents of the various species and genera. Polyphenolic presence in *Thamnochortus insignis* Mast. has however only been inferred from chemical staining properties (Linder pers. comm.).

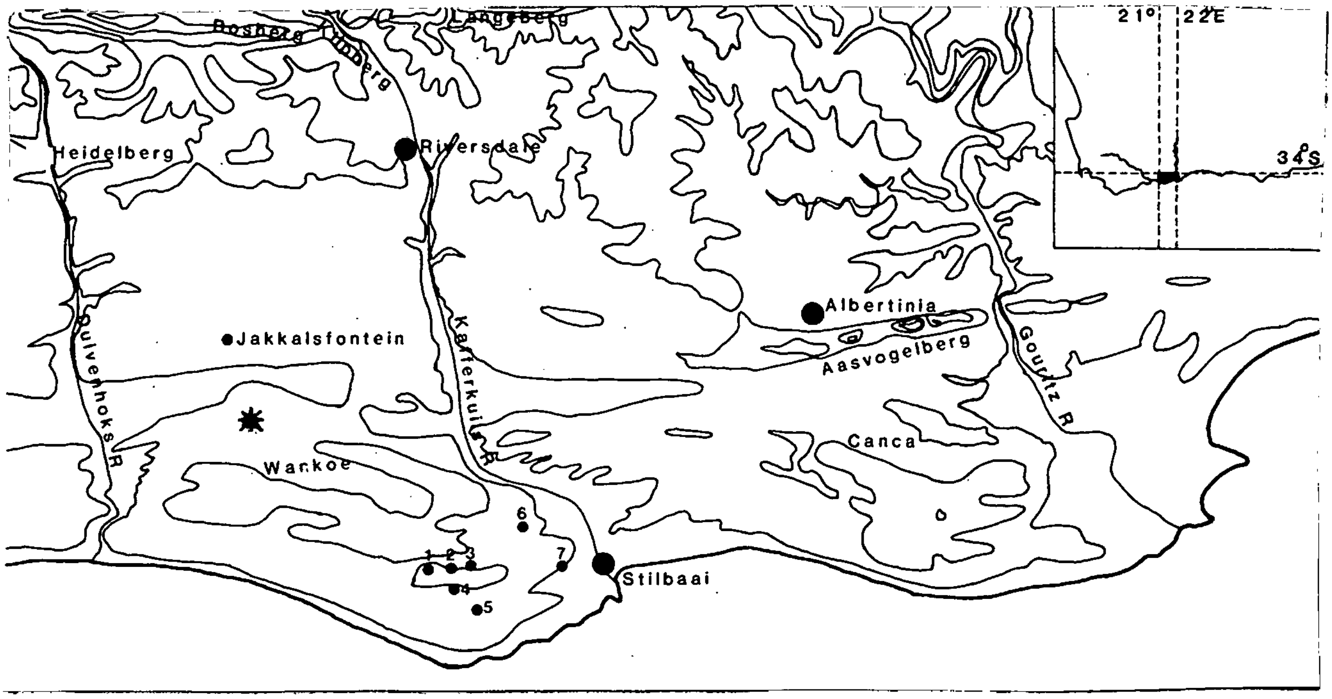
"In the past, thatching of houses was generally carried out by whatever suitable plants were in the vicinity. Recently renewed interest in thatched roofs has arisen and one Restio has emerged as economically important: the Albertinia thatching reed, *Thamnochortus insignis*" (Linder 1990). Because *T. insignis* has received considerable attention lately, as a high quality thatching reed, questions pertaining to its overall biology have been addressed. It is thought that the durability of *T. insignis* might be related to the presence of tannins (Linder and Stock pers.comm.). In turn this idea has stimulated an interest in the patterns of resource allocation to secondary compounds prevalent in *T. insignis*. Therefore the main objective of this study was to first establish if in fact secondary l.c compounds do exist in this Restio. Assuming this to be the case, the study was then designed to determine whether resource allocation to secondary compoinds in *T.insignis* ? collaborated with the ODT and "growth rate" theories. The following questions were adressed:

1) Do relative tannin-polyphenol levels vary over the different growing season (i.e. do the different aged culms exhibit different tannin-polyphenol levels)?
2) Are defenses allocated according to the risk or value of a particular tissue or organ in terms of the plant's overall fitness? How is this to be answered ?
3) How does the allocation of defense respond with varying environmental conditions; namely sunlight and nutrient availability?
4) What is the relative response of the plant's tannin-polyphenol levels in relation to defoliation?

METHODS

Study site and species description

Field work was carried out at Klipfontein farm (34° 14' S, 21° 16' E), which is approximately 25 km southwest of Riversdale (see map 1). The area lies in a rolling, hilly plain, at about 200m above sea level. The plant community studied occurs on the calcreted dune system of the Bredasdorp Formation, which is associated with colluvial sands (see plate 1). Temperatures range from a mean daily winter minimum of 12.6°C to a mean daily summer maximum of 22.0°C, averaging between 17.1 and 17.4°C for the year. The average annual rainfall in this area is 400mm. Rainfall is largely encountered in autumn when approximately 30% of the annual rain falls, decreasing slightly in winter (ca 27.5%), and more so in summer (ca 17%) (Rebelo et al. 1991). use primary references



Map 1. ✱ Study site in the vicinity of Klipfontein farm near Riversdale.

The genus Thamnochortus Berg. is a rush like xerophyte with erect, photosynthetic culms, and with the leaves generally reduced to sheaths. These plants only T. insignis! form tall tussocks up to 2,5m high (see plate ②). Thamnochortus insignis Mast. is dioecious, and the flowers are small, aggregated into spikelets and wind pollinated. The fertile culm is simple and tapers slightly from the base to the apex (Linder 1984).

Experimental design

The field work commenced on Monday, 27th May, 1991. Two 50 by 50m plots were set up in a monospecific T. insignis stand. ~~The~~ one plot was cut in June while the other was left uncut. Studies were conducted in the uncut plot. Ninety six plants, 48 of which were female and 48 male, were randomly chosen using a random number table. From these plants, 16 were again randomly chosen (8 females and 8 males) for each of the treatments. The replicates were subjected to the following treatments:

1) Control - not subjected to any form of treatment. The culms, of 3 different age groups, were clipped within each individual plant (to determine defence levels within different aged culms). These were defined as the sprouts being year 0, the flowering culms year 1, and the culms from the previous season as being year 2.

!
not all culms.
(only 4 culms
per plant have
being considered
to be the control)

Plate 1. *Thamnochortus insignis* communities studied at Klipfontein farm.

Plate 2. *Thamnochortus insignis* tussock. Height approximately two meters.

2) Fertilized - 35g of NPK fertilizer (ratio of N:P:K = 3:2:1) was sprinkled around plants in a 0,5m radius.

3) Defoliated - all the culms were clipped leaving approximately 20cm of plant material above ground.

4) Defoliated as in treatment no.3 and fertilized as in treatment no.2.

5) Shaded - tents were constructed with metal rods and 80% shade cloth. The rods were 3m long and 1cm wide, therefore allowing a certain amount of flexibility. Three rods were bound together near the apex with strong plastic coated wire. This served as the central axis from which the rods could be manipulated into the desired width, according to the size of the plant. The rods protruded approximately 25cm above the axis (see plate 3) . The shade cloth was cut into 2 x 2m squares and pushed through the protruding rods and secured with flexible wire. It was then pulled around the poles and tied down with the wire. The tents were then assembled over the chosen plant and the rods pushed in as far as possible (ca. 0,5m). The finished structures closely resembled teepees in appearance (see plate 4).

Plate 3. Method showing frame construction of the shade treatment teepees.

Plate 4. Teepee structure shading plant.