The Response of Heather (Calluna Vulgaris) to Shade and Nutrients--Predictions of the Carbon-Nutrient Balance Hypothesis

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The response of heather (*Calluna vulgaris*) to shade and nutrients — predictions of the carbon-nutrient balance hypothesis

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Summary

1. Two treatments, shade and a compound fertilizer (N, P, K, Ca, Mg) were applied in the field to heather (*Calluna vulgaris*) in a factorial design with two levels (presence or absence) of each treatment. Treatments were designed to mimic the changes in shade and soil nutrients occurring with incursion of birch woodland to heather moorland and were continued for two growing seasons.

2. The resulting morphological and chemical changes to *Calluna* were measured, the latter to test some of the premises of the carbon-nutrient balance hypothesis.

3. Shading resulted in etiolation of shoots, lower shoot densities and almost complete cessation of flowering. Overall lower shoot weights were due solely to reduced flowering. The concentration of total nitrogen increased whereas lignin, total phenolics and condensed tannins, all carbon-based constituents, decreased in the green shoot material with application of shade.

4. Application of fertilizer led to increased flowering per shoot and a greater proportion of flowering shoots. The concentration of total nitrogen in the non-flowering component of the shoots increased whereas that of lignin decreased. Unlike lignin, which is a structural polyphenolic, there was no significant decrease with addition of fertilizer, in total phenolics and condensed tannins. The results are hence not wholly consistent with the decrease in carbon-based secondary metabolites which is predicted by the carbon-nutrient balance hypothesis.

Key-words: *Calluna*, carbon-nutrient balance, nutrients, shade, succession

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Introduction

The carbon-nutrient balance hypothesis was proposed to explain the allocation of a plant's resources to putative 'anti-herbivore defenses' (Bryant, Chapin & Klein 1983). This hypothesis states that under nutrient-poor conditions, such as are often found in boreal ecosystems, woody plants have inherently slow growth rates which restrict their capacity for rapid growth beyond the reach of herbivores and for replacement of the tissue removed by them. These conditions favour the evolution of constitutive carbon-based rather than nitrogen-based chemical defenses. As applied to boreal forest trees, the hypothesis suggests that early-successional species occupying disturbed or more-fertile ground are selected to evolve defenses during their juvenile phases, when they are most susceptible to vertebrate browsing. Several tests of the hypothesis have been made using boreal tree species (Larsson *et al.* 1986; Bryant 1987; Bryant *et al.* 1987; Rousi, Tahvanainen & Uotila 1991). Here we examine patterns of allocation of resources to growth and carbon-based secondary compounds using an early-successional dwarf shrub, heather (*Calluna vulgaris* L. Hull). Although the ecological circumstances of *Calluna* are similar to early successional boreal trees, its low growth form renders it vulnerable to vertebrate herbivory throughout its lifespan. The robustness of the C/N hypothesis to other growth forms can thus be examined.

Many areas of moorland in the UK are heather-dominated vegetation types, which are plagioclimax communities maintained by fire and grazing. Heather is slow growing and occupies nutrient-poor acidic soil types in open situations which are not light-limited. In the absence of fire and with low levels of grazing, heather may be replaced by a successional sequence leading to birch (*Betula* spp.) or Scots pine (*Pinus sylvestris* L.) woodland (Miles & Young

1980; Miles 1985). The incursion of birch into areas of heather moorland leads to reduced light for the ground flora (Gardiner 1968; Miles & Young 1980; Hester 1988) and increased soil nutrient availability; the carbon:nutrient ratio decreases as succession proceeds. The consequences of these factors for the growth and flowering success of heather have already been established using field manipulations of the physical environment (Hester 1987, 1988; Hester, Miles & Gimingham 1991). Such changes in the physical environment are also expected to influence the allocation of resources to plant secondary compounds in heather. This study aims to quantify their effect on the chemical composition of heather and the interrelationships between allocation to carbon-based secondary compounds and growth and reproduction.

When considering phenotypic responses of plants to nutrient and carbon limitation, the carbon-nutrient balance hypothesis incorporates a major postulation which leads to many of the specific predictions as a result. The postulation is that in comparison to photosynthesis, growth is more strongly affected by nutrient stress. Under normal circumstances in low-nutrient environments, carbon is relatively 'cheap' (Bryant *et al.* 1983), being fixed in excess of its requirements for new growth. It is hence used in the synthesis of carbon-based secondary compounds.

Here, we test two predictions arising from the above assumptions:

1. When the nutrient levels in the environment increase, the growth and/or reproduction of *Calluna* increases and its relative allocation to carbon-based defenses decreases.

2. When photosynthesis is limited by shading, *Calluna* allocates relatively less carbon to carbon-based secondary compounds.

We manipulated these two physical variables which change with successional stage, and investigated their effects and the interactions between them.

Materials and methods

STUDY SITE

The study site was at Tulchan near Advie, Speyside, Highland Region (National Grid reference NJ 154 373). It consisted of an area of open south-east-facing heather-dominated moorland at 260 m a.s.l., which was last burnt *c.* 20 years ago. Adjacent to the site was an area of colonizing birch (*Betula pendula* Roth.). Low numbers of European rabbits (*Oryctolagus cuniculus* L.) were present in the woodland only. The only other herbivores in the area were low densities of red grouse (*Lagopus lagopus scoticus* Lath.) and roe deer (*Capreolus capreolus* L.). On the moorland less than 2% of *Calluna* shoots was grazed throughout the study period.

EXPERIMENTAL DESIGN AND TREATMENTS

The experiment consisted of a two-by-two factorial design with two levels (presence or absence) of the factors shade and fertilizer. The design was replicated six times, with each replicate containing a shaded (S^+F^-), shaded and fertilized (S^+F^+), unshaded and fertilized (S^-F^+) and a control (S^-F^-) plot. Replicates were grouped in pairs in each of three blocks.

The shading treatment was applied on six 1·5-m × 1·5-m patches of moorland using Lobrene KX02 shade netting (Lows, Dundee, Scotland) mounted on a timber frame 0·5 m high and anchored with guy ropes. The netting, which reduces light equally in all wavelengths (A.J. Hester, personal observation), permitted 12·25% light (PAR) penetration which is equivalent to that found under a dense young birch stand (Hester *et al.* 1991). Within each frame two 50-cm × 50-cm quadrats were marked, one of which was treated with fertilizer. The specially formulated compound fertilizer provided nitrogen in different forms plus other minerals (P, K, Ca, Mg) calculated to reflect soil nutrients under a developing birch stand (see Hester *et al.* 1991). Adjacent to each shaded patch was an unshaded patch of the same dimensions, also containing a fertilized and an unfertilized quadrat. The period of treatment covered two growing seasons, with shading being applied from by May to November in 1985 and 1986, corresponding to the presence of birch leaves. Fertilizer was applied twice per year, in June and August.

SAMPLING, CHEMICAL AND DATA ANALYSIS

The vegetative characteristics of the heather were sampled in September 1986. The parameters measured were the total number of long shoots (long shoot is defined as current seasons shoot extension on the main stem; Mohamed & Gimingham 1970; Gimingham 1972) and number of long shoots in flower. The numbers of flowers per flowering shoot were counted on 10 flowering shoots per plot, although in some cases under the shaded treatments, fewer than 10 were available. In November 1986, samples of 20 long shoots per plot were collected and the length and dry weights of the current season's vegetative growth and flowers were determined after separation and oven drying at 85 °C. Floral vigour was calculated as the percentage of floral mass in total shoot mass.

A further sample of shoots was collected in November 1986 and green shoot material and flowers were separated, freeze-dried and milled to pass a 1-mm sieve. Samples were anlaysed for total nitrogen by a kjeldahl technique, acid detergent fibre (ADF) and lignin by the methods of Van Soest (1963), condensed tannins by the proanthocyanidin method

(Mole & Waterman 1987) and total phenolics (Price & Butler 1977). Condensed tannins were compared with a quebracho standard provided by Dr A. Hagerman and purified according to a modification of the Asquith & Butler (1985) method (A. Hagerman, personal communication). The results of the total phenolics assay were compared with a (+)-catechin standard. Both condensed tannins and total phenolics are expressed as the equivalent percentage of standard per dry matter of sample, although this, should not be taken to imply that the samples contain either of the standards.

Data were analysed by a factorial analysis of variance (ANOVA) design nested within blocks so as to minimize any possible effects of small-scale variation between plots. There were numerous zero values for floral vigour, and ANOVAS were carried out on arcsin-transformed proportions.

Results

SHOOT MORPHOLOGY AND FLOWERING SUCCESS

The analysis of data from individual shoots shows that shade significantly decreased and fertilizer significantly increased total dry weight of shoots. The interaction term was significant ($P < 0{\cdot}05$), indicating that the effect of fertilizer was to increase shoot weight more under light conditions, than under shade (Table 1). These effects were due primarily to the inhibition of flowering by shade (see Hester 1987). The number of flowers per flowering shoot and the proportion of long shoots that flowered were significantly reduced by the shade treatments (Table 1). Although the mass of flowers per shoot and per unit area increased with fertilizer application, the increase was in proportion to the increase in total shoot mass; hence floral vigour was unaffected by fertilizer (Table 1). Overall, shading reduced and fertilizer increased the biomass production of shoots (Table 1) and this was due predominantly to the effects of the treatments on flowering. Although neither factor significantly affected the biomass of green shoot material of the individual shoots, shading significantly reduced the number of long shoots produced per area, resulting in a net reduction of total green biomass (Table 1).

CHEMICAL COMPOSITION

Chemical analysis of the long shoots with flowers removed shows that both shade and fertilizer increased total nitrogen concentration (Table 2). Neither of the main effects was significant for ADF, but the interaction term was significant indicating that when applied alone both shade and fertilizer reduced ADF concentration, but they did not do so when applied in combination. Both shade and fertilizer reduced shoot lignin concentration but the effect of fertilizer was reduced in the presence of shading (Table 2). Condensed tannins and total phenolics were significantly reduced by application of shade. Addition of fertilizer reduced both condensed tannins and total phenolics, but not significantly to (Table 2).

Shading inhibited flowering almost completely and hence insufficient flower material was available to permit chemical analyses of flowers from the shade treatments. Although fertilizer increased both weight of flowers per shoot and total biomass of flowers produced, neither floral vigour nor the concentrations in the flowers of either condensed tannins (S^-F^-, 47·9% DM; S^-F^+, 49·1% DM,

Table 1. The effects of shade (S) and fertilizer (F) on shoot growth and flowering success of *Calluna*. All shoot-based measurements (except no. of flowers per flowering shoot) are from samples of 20 shoots within each of six plots per treatment. All area-based measurements refer to the means of six plots per treatment. The significance of the *F* values are given as $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$. SED = SED of interaction. Unlike superscripts within rows indicate a significant ($P < 0{\cdot}05$) difference between means (LSD test, Snedecor & Cochran 1980)

	Treatments				ANOVA			
	S^-F^-	S^-F^+	S^+F^-	S^+F^+	S	F	S × F	SED
Lengths of long shoots (mm)	45·2^{a}	53·2^{ab}	53·7^{ab}	61·7^{b}	**	**	—	3·60
Shoot dry weights (mg)								
total	36·5^{b}	50·4^{c}	18·1^{a}	20·7^{a}	***	**	*	3·50
green material	19·3^{ab}	25·7^{b}	18·1^{a}	20·5^{ab}	—	—	—	2·99
flowers	17·1^{b}	24·6^{c}	0·0^{a}	0·1^{a}	***	**	**	1·50
Floral vigour (%)	46·9^{b}	49·8^{b}	0·0^{a}	0·6^{a}	***	—	—	2·80
No. of flowers per flowering shoot	19·4^{b}	17·0^{b}	7·9^{a}	8·4^{a}	***	—	—	2·55
Number (m^{-2}) of								
long shoots	5973^{b}	6227^{b}	3791^{a}	3579^{a}	***	—	—	610·9
flowering long shoots	5767^{b}	6060^{b}	31^{a}	26^{a}	***	—	—	372·1
Biomass ($g\,m^{-2}$)								
total	220·2^{b}	310·7^{c}	72·0^{a}	74·2^{a}	***	**	*	25·3
green material	118·1^{ab}	157·5^{b}	72·0^{a}	73·5^{a}	***	—	—	20·6
flowers	102·0^{b}	153·1^{c}	0·0^{a}	0·7^{a}	***	**	**	10·8

Table 2. The chemical composition (% Dry matter) of the non-flowering portion of the current season's growth. Symbols and superscripts as for Table 1

	Treatments				ANOVA			
% Dry matter	S^-F^-	S^-F^+	S^+F^-	S^+F^+	S	F	S × F	SHD
Total nitrogen	1·15^{a}	1·46^{b}	1·80^{c}	2·06^{d}	***	***	—	0·061
ADF	37·7^{b}	35·0^{a}	34·9^{a}	36·1^{ab}	—	—	**	0·94
Lignin	22·5^{c}	19·4^{b}	17·6^{ab}	17·1^{a}	**	**	*	0·78
Condensed tannins	20·8^{b}	19·3^{b}	16·2^{ab}	13·7^{a}	**	—	—	2·07
Total phenolics	6·0^{b}	5·8^{b}	5·4^{ab}	4·8^{a}	**	—	—	0·27

SED 3·68) or total phenolics (S^-F^-, 5·4% DM; S^-F^+, 5·4% DM, SED 0·07) were affected. It should be noted that flower pigments consist of high levels of procyanidins which give a strong colour reaction under the conditions of the proanthocyanidin assay (Harborne 1988), and may result in disproportionately high apparent levels of condensed tannins.

Discussion

MORPHOLOGICAL AND CHEMICAL RESPONSES OF *Calluna* THEIR ROLE IN SUCCESSION

The imposition of the treatments for two growing seasons means that any effects were unlikely to be buffered by stored reserves from a previous season (Elmqvist *et al.* 1991). There was a profound effect of shade on the growth and morphology of *Calluna*, the main effects being almost total cessation of flowering, production of fewer long shoots and elongation of shoots without increase in their mass. The etiolation response is interpreted as an attempt to avoid shade imposed by neighbouring plants of the same or other species, and is consistent with observations of the importance of light intensity in limiting the rate of photosynthesis in *Calluna* (Grace & Woolhouse 1970). The effect of low light levels *per se* was to reduce considerably the lignin, condensed tannin and phenolic concentrations of the long shoots, indicating a diversion of scarce carbon resources away from these carbon-based components. The addition of fertilizer also reduced lignin concentration and, although the effects on the condensed tannin and total phenolics were in a similar direction, they were not significant. These conditions of low light intensity and increased nutrient availability are those that pertain during the successional incursion of birch into heather moorland. In this experiment they also resulted in increases in total nitrogen content of the heather; the form of this nitrogen may be as protein, accumulated amino acids or nitrates (Waring *et al.* 1985; Lahdesmaki *et al.* 1990).

These changes in the chemical composition of heather shoots would make them a more favourable food source for a range of herbivores. Herbivores including red grouse, red deer (*Cervus elaphus* L.) and mountain hares (*Lepus timidus*) have been shown to select *Calluna* which is high in nitrogen (Miller 1968; Moss 1977; Moss, Welch & Rothery 1981) or a diet which is low in phenolics (Iason & Waterman 1988). *Calluna* phenolics have been implicated in reducing digestibility of heather by sheep (Milne 1974) and in causing sodium imbalance in mountain hares (Pehrson 1983). Thus it may be hypothesized that birch colonizing a heather moor predisposes the heather to a greater risk of attack by herbivores, which may then hasten the disappearance of heather from the ground layer, thus influencing the successional vegetation changes. However, because birch woodland would only develop under conditions of low to moderate grazing intensity, this scenario is unlikely. Only if higher densities of herbivores were found in an area of woodland after its initial establishment phase might they have a role in the subsequent successional process. This circumstance may occur in association with greater availability of shelter or general nutrient enrichment of the area by *Betula*.

THE PREDICTIONS OF THE CARBON-NUTRIENT BALANCE HYPOTHESIS

The initial premise of the carbon-nutrient balance hypothesis, that application of shade would reduce carbon-based secondary compounds was borne out, as shading alone reduced both condensed tannin and total phenolic concentrations in the shoots. Several tests have confirmed that when light or carbon dioxide is limited, the rate of photosynthesis is reduced and, as fixed carbon becomes limited, production of carbon-based secondary metabolites is reduced (Larsson *et al.* 1986; Bryant 1987; Mole, Ross & Waterman 1988; Nicols-Orions 1991).

This study is in agreement with others (Larsson *et al.* 1986; Bryant 1987) in which treatments with higher levels of nutrients did not consistently lead to lower concentrations of total phenolics or tannins. Bryant (1987) found a reduction of phenolic compounds, measured by several different means, when fertilizer treatments were applied without artificial clipping of willow (*Salix alexensis* L.) but no effect with artificial clipping. Both the studies cited support the carbon-nutrient balance hypothesis with regard to the effect of reduced light in reduction of carbon-based secondary compounds, but with respect to the effect of fertilizer they do not do so unequivocally. Furthermore, studies of boreal tree species have also failed to show a consistent decrease in shoot terpenoids, another putative carbon-based defense system, in response to fertilizer application (Hiltunen, von Schantz & Loyttyniemi 1975; Gershenzon 1984; Bjorkman, Larsson & Gref 1991). In these cases, positive associations between nutrient addition, growth and the carbon-based secondary metabolites in the shoots could be attributed to the expansion of the terpenoid-containing resin ducts with tissue growth (Bjorkman *et al.* 1991). However, if there is to be a net increase in terpenes in the plant as a whole, there must be some increase in synthesis rather than the predicted decrease. In contrast, several other studies of both plants and cell cultures do provide support for the second of the tested premises, i.e. phenolic levels would decrease with increased nutrient supply or that phenolics would increase with nutrient stress (Gershenzon 1984; Waring *et al.* 1985; Bryant *et al.* 1987; Nicols-Orions 1991).

The positive growth and flowering response of *Calluna* to fertilizer confirms that nutrients are a likely limiting factor under field conditions. However, it does not necessarily follow from this that allocation of resources to carbon-based secondary compounds is reduced when growth increases (Rousi *et al.* 1991). The present data and the inconsistencies between the other studies lead us to question the generality of the carbon-nutrient balance hypothesis. The relationship between nutrient levels and secondary 'defenses' is central to the hypothesis. Low nutrient availabilities and the high levels of carbon-based 'defenses' presumed to result from this, are a feature of the boreal systems for which the hypothesis is proposed (Bryant *et al.* 1983). The carbon-nutrient balance hypothesis and the more-generalized resource-limitation hypothesis (Coley, Bryant & Chapin 1985) provide useful descriptions of the evolutionary pressures that may operate to produce different levels and types of chemical defenses, particularly between plant species. However, they are limited in their ability to predict phenotypic responses to environmental changes. This may be achieved by a fuller understanding of the costs and benefits of investment in secondary compounds in relation to growth and the relationships between photosynthesis, growth and synthesis of secondary compounds, and their regulation, under different levels of nutrient availability.

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