Autoinhibition of germination and seedling establishment by leachate of Calluna vulgaris leaves and litter

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Abstract: Allelopathic effects of Calluna vulgaris have been extensively studied, but little attention has been given to the dynamics of phytotoxicity during the decomposition of its litter. Strong evidence from vegetation dynamics and management strategies indicate that Calluna litter inhibits its own seed regeneration. This work investigates the hypotheses that Calluna tissues are autotoxic and that decomposition processes moderate this effect. Water extracts of fresh Calluna shoots, litter and surface soil from a burned stand were used in both aerobic and anaerobic conditions to test for phytotoxicity in Lepidium sativum and Calluna vulgaris seeds and seedlings.

Phytotoxicity varied for undecomposed Calluna materials with the following rank: shoots >> litter > burned soil. Moreover, phytotoxicity on Lepidium and Calluna of all tested materials sharply decreased during aerobic decomposition, but it remained high in anaerobic conditions. This paper confirms that Calluna shoots and litter are autotoxic, and demonstrates clear patterns related to decomposition processes. The potential consequences for vegetation dynamics are discussed.

Introduction

Plant litter has been reported to induce both positive (Facelli and Pickett 1991, Xiang and Nilsson 1999) and negative (Wedin and Tilman 1993, Bergelson 1990, Singh et al. 1999) influences on the regeneration and growth of plants. These effects have been attributed to different mechanisms such as: physical impediment (Wedin and Tilman 1993), reduced light penetration and changes of the Red/Far Red ratio (Schimpf and Danz 1999), predation activity (Facelli 1994), and release of allelochemical compounds during decomposition (Van der Putten et al. 1997, Blum et al. 1999, Armstrong and Armstrong 2001). The allelopathic effects of plant litter have been extensively studied (reviewed in Rice 1984), but few studies have dealt with the dynamics of phytotoxicity during the decomposition process (Harper 1977). Changes over time of both quantity and composition of allelochemicals occur by sorption and polymerisation by soil organic matter and clay minerals (Makino et al. 1996) and chemical transformation by microorganisms (Blum et al. 1999). This can either increase or decrease the phytotoxicity of decomposing plant litter (An et al. 2001). Phytotoxicity dynamics have been documented for several crop species in aerobic conditions (Patrick et al. 1963, Chou and Lin 1976) but only in few studies in natural plant communities (Jäderlund et al. 1996, Bonanomi et al. 2005a). Anaerobic conditions have been reported to produce stronger and more persistent phytotoxic levels (Patrick 1971, Armstrong et al. 1996). The highest levels of inhibition have been reported in the early stages of decomposition followed by a decline in phytotoxicity (Cochrane 1948, Jäderlund et al. 1996, Bonanomi et al. 2005a).

The allelopathic effect of ericaceous species such as Erica tetralix L., Erica mediterranea L., Erica scoparia L. and Calluna vulgaris L. is well know and has been related to the release of hydrolysable phenolic compounds (Gimingham 1972, Ballester et al. 1977, Rice 1984, Hille
and Ouden 2005). However, less attention has been given to the role of decomposition in affecting the phytotoxicity of litter in these species. Ample evidence suggests that Calluna vulgaris litter can inhibit its own regeneration in field conditions. In fact, despite the abundance of seeds and light availability at ground level (Gimingham 1972), germination is restricted and Calluna seedling survival is very poor in the building to degenerate phases of its life cycle (Watt 1947, de Hullu and Gimingham 1984) when there is high leaf shedding and litter accumulation. Moreover, Calluna regeneration from seed has been reported to be negatively affected by addition of Calluna litter whereas it can be increased by litter removal (Mallick and Gimingham 1985) and strongly enhanced by fire events (Gimingham 1972).

This study investigates the allelopathic effect of aboveground material (shoots, litter and top soil) on Calluna vulgaris seed germination and seedling survival and describes the changes in phytotoxicity during the decomposition process in aerobic and anaerobic conditions. The implications of this work, conducted under controlled and standard conditions, for the dynamics of natural communities are discussed.

**Methods**

All material was collected in April 2002 in the Pentland Hills Regional Park (Midlothian, Scotland) from an uneven-age stand of Calluna vulgaris. The following materials were collected: 1) undecomposed shoots of Calluna; 2) aboveground Calluna litter; and 3) top soil (first 2 cm) from a stand burned one year previously. Plant materials and soil were dried (40°C for 5 days) and then stored at room temperature. Calluna seeds were collected in the field from flowers remaining on the plants (n° plants > 20) and stored in dry conditions at room temperature until bioassay.

Dry materials (shoots, litter and soil) were dispersed in distilled water (50 g dry weight per litre) at 25 °C. Decomposition of these aqueous suspensions was carried out in 2-litre flasks at 20±2 °C for 15 days. Aerobic conditions were obtained by pumping air into the solution, whereas closed flasks generated anaerobic conditions. Distilled water was added to the flasks to compensate for evaporation losses. A microbial soil inoculum was prepared with suspension of 10 g of heathland soil in 100 ml water and added (10 ml/flask) to the extracts to improve the start up of the decomposition processes. This experimental method for decomposition processes is rapid and easily reproducible (Zucconi et al. 1981, Bonanomi et al. 2005a). The aqueous suspensions are considered to resemble natural conditions in the soil that the soil microbial community is in the soil solution or living in the thin water film around or inside soil particles and aggregates (Stotzky 1997, Nannipieri et al. 2003).

Samples of the aqueous suspensions were extracted before the addition of the soil inoculum, 24 hours after the start of the incubation period and after 15 days. They were centrifuged (4300 rpm for 10 minutes), sterilised (microfiltration with a 0.22 µm pore filter) and stored at –20 °C until bioassay. Electrical conductivity and pH were measured. The original solution and two further dilutions with distilled water were prepared for bioassay giving concentrations of 5%, 1.5% and 0.5% on a dry weight basis.

Seed germination tests were carried out with Calluna and Lepidium sativum L.. The latter is recognized as a very sensitive bioassay and was utilized as a standard comparison (Zucconi et al. 1981, Heil et al. 2002, Gehring et al. 2003). Bioassays were also carried-out with Calluna seedlings.

The first germination experiment was carried-out with Lepidium seeds to evaluate the effect of undecomposed extracts of Calluna shoots, litter and burned soil. The experimental design of germination test of Lepidium included 10 treatments (3 materials x 3 concentration levels plus control with distilled water). Ten seeds of Lepidium were place on filter papers with 4 ml of the test solution in 15 replicate 9-cm Petri dishes for each treatment (1500 seeds in total).

The second germination experiment was carried-out with Calluna seeds to evaluate the autotoxic effect of undecomposed and decomposed extracts of Calluna shoots, litter, and burned soil. This experiment comprised 10 treatments (3 materials x 3 decomposition conditions, all at high concentration level, plus control with distilled water). Twenty seeds of Calluna were placed on filter paper with 4 ml of the test solutions in 15 replicate 9-cm Petri dishes for each treatment (3000 seeds in total).

Petri dishes in both experiments were laid out in a totally randomised design in a growth chamber and maintained at constant temperature (25 °C; day/night ratio 16/8 hours). Germination percentage and root length of Lepidium seedlings was measured 48 hours after the beginning of the experiment. Calluna germination is a much slower process (Gimingham 1972) and germination percentage was measured 25 days after the beginning of the experiment.

The bioassays with Calluna seedlings were carried-out to evaluate the autotoxic effect of undecomposed and decomposed extracts of Calluna shoots, litter, and burned soil. Calluna seedlings with five pairs of leaves (approxi-
mately 40 days old) were used (shoot and root length ranged between 15-20 mm and 5-10 mm respectively). Seedlings were prepared by natural germination from top soil collected in the burned Calluna stand. Seedlings were watered daily with distilled water. For the experiment each seedling was placed in a 1.5 ml eppendorf tube (Eppendorf) with 1 ml of distilled water. After 24 hours the distilled water was replaced with the test solution (1 ml for each tube) and then the solution was replaced every 24 hours to avoid further decomposition. The experimental design provided for ten treatments (3 materials x 3 decomposition conditions plus control with distilled water). Twenty replicate seedlings were used for each treatment giving a total of 200 plants. The plants were placed in a growth room at 25°C, 90% relative humidity and day/night ratio 16/8 hours. The numbers of healthy, wilting and dead seedlings were counted every day for 96 hours.

Two-way ANOVA was applied to test the main effect and interaction of materials (shoots, litter and top soil) and extract concentrations on germination and root length of Lepidium. Two-way ANOVA was also performed to test the main effects and interactions of conditions of decomposition (aerobic and anaerobic) and plant material (shoots, litter, top soil) on Calluna germination.

Results

Lepidium germination was close to 100% in all treatments with no significant treatment effects (data not shown). Lepidium root length was significantly affected by the materials, concentration and their interaction (Two-way Anova: P values always < 0.001). Root extension was concentration dependent in shoots and litter extracts with greater inhibition at higher concentrations. Shoots extracts were the most phytotoxic followed by litter, whereas the burned soil extracts did not show any evidence of phytotoxic effect (Fig. 1).

Calluna germination was significantly affected by the conditions of decomposition (two-way ANOVA, F = 43.6; P < 0.001), type of materials (F = 41.1; P < 0.001) and the interaction was also significant (P < 0.001). Germination was negatively affected by undecomposed shoots whereas extracts of litter and top soil did not show statistically significant differences from the control (Fig. 2). Decomposition in aerobic conditions neutralised the phytotoxic effect from both shoots and litter, but anaer-
Phytotoxic compounds are variously released, produced, transformed and destroyed during the decomposition processes of decaying plant material (Blum et al. 1999). The initial phase of decomposition basically consists of plant tissue comminution and subsequent release of the cell contents. Calluna shoots resulted in the most phytotoxic material, whereas the phytotoxicity of Calluna litter and burned soil was much lower (Figs. 1, 2 and Table 1) implying that the phytotoxic constituent of the living shoot degrades over time.

Aerobic decomposition significantly reduced the phytotoxicity that remained high, or even increased, during anaerobic decomposition (Figs. 1, 2 and Table 1). These contrasting effects of aerobic and anaerobic decomposition are consistent with previous studies on crop species (Patrick 1971) and support the hypothesis of microbial activity being the driving factor of litter phytotoxicity (Zucconi et al. 1981). Variations of pH and electrical conductivity of the extracts cannot explain the observed effects because control-levels of germination were found in solutions with both high and low pH values and extreme values were associated with both very phytotoxic and non-toxic extracts.

Studies of allelopathy in field conditions are rare and their interpretability has been limited by the lack of comparative experimental bioassays (Inderjit and Callaway 2003). A major review of allelopathy pointed out how plant-produced phytotoxic compounds would be rapidly degraded by the soil microbial activity into non-toxic molecules, thus reducing the expected impact on plant population dynamics (Harper 1977). The results of this work showed that phytotoxicity can persist in anaerobic conditions. This is likely to interact with other stresses of the anoxic environment, such as oxygen deficit (Callaway and King 1996) and the presence of inorganic toxic compounds, e.g., acid sulphite, ferric and nitrite ions (Marschner 1995). Anaerobic conditions can be found locally in Calluna stands depending on climatic condition, soil drainage and microtopography. On the other hand, our laboratory results showed that in aerobic conditions autotoxicity is limited to the early phases of shoot decomposition. In fact, Calluna litter can accumulate in deep layers (e.g. 1500-1700 g m⁻² in 15–20 year-old stands, Chapman 1967), this can produce a mechanical impediment for seedling emergence and development (Miller 1996, Gimingham 1972). The observed absence of phytotoxicity of soil after fire, is compatible with the hypothesis of combined mechanical and autotoxic litter effects.

Litter autotoxicity (Singh et al. 1999) has been reported for several crop and natural species (McNaughton 1968, Van Der Putten et al. 1997) and negative plant-soil feedback (Van der Putten et al. 1993, Bever 1994, Kliromenos 2002, Bonanomi and Mazzoleni 2005, Bonanomi et al. 2005b) has been considered for its effects on successional dynamics and species coexistence (Bever 2003, Bonanomi et al. 2005c). This study added further evidence to the importance of a negative feedback between
plant and soil as a factor explaining cyclical vegetation dynamics, such as the well known degenerative-regenerative phases of natural Calluna stand (Watt 1947, Barclay-Estrup and Gimingham 1969, Gimingham 1988).

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References


