Root Senescence in Red Clover (*Trifolium Pratense* L.)

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Introduction Legume root systems form a mosaic of living, ageing and dead roots and nodules. The balance between these stages alters during plant development. Stressful events (drought, temperature change, reduced carbon supply, etc.) disturb the balance (Butler *et al*., 1959). Effects of root and nodule death on soil structure, composition and leaching and on plant persistency are understood poorly. Plants with differing senescence patterns are useful tools to study these effects. Molecular studies of root senescence need detailed knowledge of the process and timing of root senescence and death. Biochemical and histochemical markers of senescence were used to generate preliminary results of the effects of reduced carbon input, temporary (by defoliation, D) or permanent (by defoliation and shading, DS) on red clover shoot survival and root death.

Materials and methods In a controlled environment (20°C/15°C light/dark; 16h photoperiod; 400uM/m/sec; nitrogen-free nutrients), nodulated clover seedlings cv. Milvus were grown for 7 weeks in Agsorb. At day 0, plants were either defoliated (D), defoliated and heavily shaded with black polythene (DS), or left intact (control). Shoot re-growth, root death index (RDI, based on quantitative Evan's Blue staining (Baker & Mock, 1994)) and root catalase activities (Doulis *et al*., 1997) were measured (3 replicates/treatment) at 0, 7, 14 and 21 days. After 21 days, DS plants were grown for a further 14 days in the light before assessment of RDI. Statistical analysis was by ANOVA (Genstat).

Results Red clover plants recovered from temporary (D) but not permanent (DS) reduction in carbon supply (Figure 1A). Quantitative differences in RDI were not significant between any of the treatments for the first 14 days, indicating that similar proportions of cells of these roots were still alive (Figure 1B). By 21 days, DS roots were significantly more strongly stained ($p<0.05$) indicating a higher level of cell death. Root catalase activity showed a similar pattern (Figure 1C); there were no significant differences in catalase activity between control, D and DS plants at 7 or 14 days. Root catalase activity of DS plants increased about 8-fold by day 21 ($p<0.05$). After a further 14 days growing in the light, all roots from DS plants stained strongly with Evan's Blue, resulting in OD similar to dead, control roots (Figure 1B).

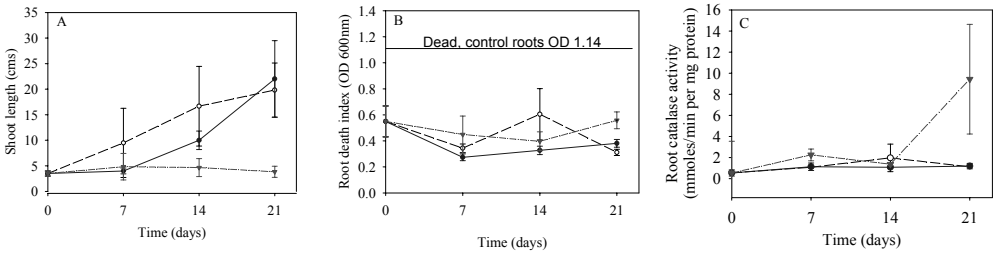

Figure 1 Effects of temporary or permanent reduction in carbon supply in red clover cv. Milvus over time. **A** Shoot growth. **B** Root death index. **C** Root catalase activity. —o— Control; —●— Defoliated (D); —▼— Defoliated and shaded (DS).

Conclusions Red clover roots survived temporary reduction in carbon supply (D) and had no change in root senescence. Permanent reduction in carbon supply (DS) caused plant death by 21 days; shoots failed to re-grow when returned to light. These data show that defoliation alone is not enough to trigger root death and provide a temporal framework for studies on differential gene expression during root senescence.

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