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Factors affecting the longevity of clover roots following shoot excision and its implications for managing N cycling in arable cropping systems

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ABSTRACT

Controlled environment experiments were conducted to investigate factors that affect the longevity of clover roots after permanent shoot excision and its implications for managing N cycling in arable cropping systems. The hypothesis tested was that root longevity is related to the initial soluble sugar concentration of the tissue and its rate of depletion after defoliation. Red clover plants were grown in either sand or soil (depending on the experiment) for eight weeks before the shoot was excised at the crown. Root cell viability and concentrations of soluble sugars, starch, amino acids and soluble phenols were determined at regular intervals for up to seven weeks after defoliation. The effects of mechanical damage to the roots, shading of the shoot prior to defoliation, soil temperature, microbial inoculation and nature of the root growth substrate were investigated. Root longevity, defined as the time taken for more than 80% of root cells to lose viability after shoot excision, varied from two weeks to over seven weeks between treatments. Soluble sugar and starch concentrations declined after shoot excision. Treatments affected the initial concentration and rate of depletion of soluble sugars, but had little effect on starch concentrations. Amino acid concentrations increased temporarily after shoot excision before declining; in most cases the decline coincided with the loss of cell viability. Pooling data from the different experiments indicated a threshold bulk tissue sugar concentration of 24 mg g DW^{-1} below which cell viability declined. There was a significant positive relationship (r^2 0.93) between the initial sugar concentration and root longevity when roots were left undisturbed after shoot excision. When roots were disturbed and cut into fragments, the rate of sugar depletion was accelerated and root longevity reduced compared to undisturbed roots. The results suggest that tillage to damage the root system would be a more effective method of enhancing the rate of root senescence, and by inference the early release of N, than defoliation. The data can be used to refine models of nutrient cycling in arable systems to account for the period of root senescence prior to post-mortem decomposition.

1. Introduction

Global food demand is predicted to double from 2000 levels by the year 2050 increasing the requirement for N in arable production systems (Tilman et al., 2002). As a result of the high energy and economic costs associated with the manufacture of N fertilizer it is unlikely that this greater requirement can be sustained solely through the increased use of inorganic sources of N. Biological N_2 fixation provides an alternative means of supplying some of the N required by arable systems, but managing this form of N supply is more challenging (Thorsted et al., 2006a; Launay et al., 2009). It is important to ensure that there is sufficient N_2 fixed, the N is made available to non-fixing crops such as cereals when their demand is greatest, and that losses (e.g. nitrous oxide emissions and nitrate leaching) from the system are minimised. Effective management requires a good understanding of the factors governing N_2 fixation and N transfer processes.

Clover is used extensively to supply N in pasture systems. Transfer of N from clover to grass species can occur via root exudation, the death and decay of nodules and root tissues and the digestion of root and shoot tissues by grazing animals and subsequent excretion of N (Brophy and Heichel, 1989; Martin et al., 1991; Høgh-Jensen and Schjoerring, 1997; Murray and Hatch, 1994). There is also interest in using clover to supply N within arable systems. Clover can be undersown into existing cereal crops or grown as a bi-crop (inter-crop) where the cereal is sown into an established clover sward (Bergkvist et al., 2011; Schmidt et al., 2001; Thorsted et al., 2006a). There are a number of potential

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agronomic tools for managing the release of N from clover for use by the companion or succeeding crop. These include promoting the senescence and degradation of roots and nodules by partial or complete defoliation either mechanically or through the use of a herbicide (Thorsted et al., 2006b).

Although there is an extensive literature on the factors that regulate the rate of plant tissue decomposition the majority of studies have been conducted on dead tissues (van der Krift et al., 2001; de Graaff et al., 2010; Fujii and Takeda, 2010; Urcelay et al., 2011). Little is known about what influences the dynamics of senescence *prior* to root death and post-mortem decomposition. Hence it is difficult to predict when N may become available for subsequent plant uptake. Previous research has shown that red clover roots can survive for remarkably long periods of time after permanent excision of the shoot (Bingham and Rees, 2008). Roots grown in sand culture remained viable for as long as six weeks when left undisturbed after removal of the shoot. Moreover, significant net release of N from root tissue only occurred after cell viability had been lost (Bingham and Rees, 2008). However, it is unclear whether the same longevity might be expected under field conditions.

The physiological mechanisms regulating root senescence are not well understood. There is evidence that senescence of excised roots may be induced by sugar starvation associated with the depletion of soluble carbohydrate reserves (Brouquisse et al., 1991; Devaux et al., 2003; Bingham and Rees, 2008). The time required to deplete soluble sugar concentrations to critical levels will depend on their initial concentration in the tissue, the rate of metabolic activity (as sugars are the major substrate for respiration), and the extent to which sugar concentrations can be buffered by mobilisation of storage reserves such as starch. As such, factors that alter the tissue concentration, rate of respiration, or utilization of storage reserves might be expected to influence the longevity of the roots after shoot excision. Concentrations of soluble sugars and storage reserves are likely to depend on the crop production system adopted and, in particular, on the extent of shading by the companion crop grown with the clover. Other possible factors affecting the longevity of root tissues include the presence of pathogenic and non-pathogenic micro-organisms and the extent of physical damage to the roots. There is some evidence that disturbance of the root system at the time of shoot excision may reduce subsequent root longevity (Bingham and Rees, 2008). The aim of experiments reported in this paper was to determine the effects of several soil and plant management factors on the longevity of clover roots after permanent excision of the shoot (as might occur after tillage) and to assess their implications for managing N supply in arable systems. The senescence of excised roots considered here is distinct from the turnover of individual roots within the root systems of intact or partially defoliated plants (e.g. following grazing) where shoot growth occurs and re-translocation of N from the roots to the shoot can take place (Corre et al., 1996). The factors examined were those that might be encountered by clover in an arable cropping system; disturbance and physical wounding of the tissue, shading of the shoot prior to excision, the nature of the root growth substrate (soil compared with the sand used in previous research), the temperature regime of the root environment and the presence of a microbial inoculum. The data were used to test the hypothesis that the longevity of clover roots after permanent shoot removal is related to the initial concentration and rate of depletion of soluble sugars in the tissue.

2. Materials and methods

2.1. Plant growth

All experiments were conducted on red clover (*Trifolium pratense* L) cv Merviot. Seeds were germinated in trays of moist sand, in a growth cabinet maintained at 20 ± 0.5 °C with a photon fluence of 300–400 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) provided by quartz halogen lamps over a 16 h photoperiod. Unless otherwise stated in descriptions of individual experiments, after 14 days seedlings were transplanted into plastic horticultural root trainers (120 mm deep; 350 ml volume) (Highland Garden Supplies Ltd, Inverness, UK) loosely packed with grit sand (horticultural grade, washed sand, nominal size <5 mm). After transplanting, seedlings were placed in a growth cabinet set at a constant 20 ± 0.5 °C. Light was supplied by high intensity fluorescent lamps at a photon fluence of 500–600 μmol m^{-2} s^{-1} PAR at initial plant height over a 16 photoperiod. To reduce transpiration rate and the risk of water stress developing, a high humidity was maintained around the shoots by placing a vented clear polythene cloche over the seedlings. Plants were irrigated every Monday and Friday with a balanced nutrient solution containing all the essential macro- and micro-nutrients (the higher strength solution reported by Bingham and Stevenson, 1993). On Wednesdays, they were irrigated with deionised water. In each case sufficient solution was applied to bring the sand back to field capacity (the point at which excess solution drained from the bottom of the container). Eight weeks after transplanting, shoots were excised from the roots at the crown to prevent re-growth of foliage. The excised roots were left *in situ* until the designated time of sampling or imposition of additional treatments. The moisture status of the growth substrate was maintained by irrigating with deionised water every seven days. Experimental treatments are summarised in Table 1 and described in detail below. Clover plants were not specifically inoculated with Rhizobium species, but some small nodules developed on both sand (all experiments) and soil-grown (experiment 4, details below) roots from naturally occurring populations. Cell viability was measured on root cortical cells and tissue composition determined on nodulated root tissue, the mass of which was dominated by the root (methods described below).

2.2. Experiment 1 – mechanical damage

Sand that had been heat-treated to remove organic matter was placed into 500 ml glass Kilner jars and brought to 12 % v/v moisture using filter-sterilised, N-free, nutrient solution as described by Bingham and Rees (2008). Plants were grown as described in section 2.1 and after excising the shoot, root systems were removed from their root trainers, gently washed free of sand in deionised water, blotted dry and weighed. The roots were then either cut into 10–20 mm long fragments or left intact and layered between the moist sand within the Kilner jars. The lids were sealed and the jars

Table 1
Experimental treatments and culture conditions before and after shoot excision.

Experiment and treatment	Pre-shoot excision	Post-shoot excision
1. Mechanical damage	Sand culture	Roots transferred to culture vessel and left intact or cut into 10–20 mm lengths
2. Shading	Sand culture. 84% shading applied 14 days before shoot excision. Controls unshaded	Roots left *in situ*
3. Temperature regime & microbial inoculation	Sand culture	Roots left *in situ* and incubated at either constant 20 °C or variable soil temperature (20–15 °C day/night) each with and without microbial inoculation
4. Growth substrate	Soil or sand culture	Roots left *in situ*